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THE INFLUENCE OF TEMPERATURE AND SEASON ON THE CITRUS RED MITE (PARATETRANYCHUS CITRI) [1]

By L. L. ENGLISH, *entomologist, Department of Zoology-Entomology, Alabama Agricultural Experiment Station,* and G. F. TURNIPSEED, *entomologist, Alabama Department of Agriculture and Industries* [2]

INTRODUCTION

Several investigators have emphasized the importance of temperature in relation to the development and abundance of the citrus red mite (*Paratetranychus citri* McG.). Woodworth [3] observed as early as 1902 that there was a relationship between the population of this pest and the season of the year. Quayle [4] noted that the population of mites reached its greatest density during the spring months and largely disappeared in the summer. Boyce,[5] some years later, pointed out that infestations of this mite were distinctly limited by climatic factors and that it thrived best under cool, semihumid conditions. Quayle[6] presented a map showing that the regions of California in which the citrus mite is a serious pest are along the Pacific coast. At Los Angeles, for example, the mean monthly temperature is about 70° F. in July and August. At Riverside, where the mean monthly temperature for these 2 months is 75°, citrus mite occurs but is not an important pest. Further inland, at Lindsay, the mean monthly temperature is near 80° during July and August, and in this region the citrus mite is not known as a pest. Although the influence of temperature on the citrus red mite has been recognized, apparently no definite relationship between temperature and mite development has been established.

The experiments whose results are herein reported were carried out over a period of several years. Temperature observations, the time required for mites to develop from egg to adult, and the length of life of individual mites were recorded from 1930 to 1936, inclusive. After preliminary studies showed that a relationship existed between temperature and incubation and development, additional cultures were started in 1938 to obtain more complete data on life history, as well as further information on the variation of development with season. From these data an attempt was made to show that, although development is accelerated by increases in temperature, other factors promote population density in cool weather.

[1] Received for publication Dec. 11, 1939.

[2] Field laboratory, Spring Hill, Ala.

[3] WOODWORTH, C. W. THE RED SPIDER OF CITRUS TREES. Calif. Agr. Expt. Sta. Bul. 145, 19 pp., illus. 1902.

[4] QUAYLE, H. J. CITRUS FRUIT INSECTS. Calif. Agr. Expt. Sta. Bul. 214: [443]-512, illus. 1911.

[5] BOYCE, A. M. THE CITRUS RED MITE PARATETRANYCHUS CITRI M'G. IN CALIFORNIA, AND ITS CONTROL. Jour. Econ. Ent. 29: 125-130. 1936.

[6] QUAYLE, HENRY J. INSECTS OF CITRUS AND OTHER SUBTROPICAL FRUITS. 583 pp., illus. Ithaca, N. Y. 1938.

METHODS

The rearing cells constructed of felt and celluloid described by Newcomer and Yothers[7] were used for observing individual mites. From 1930 to 1936, inclusive, cells half an inch in diameter were used. During 1938 the size of the cells was increased to 1 inch to provide more freedom and food for the individuals. The cells were fastened to the leaves of small Satsuma trees grown in 12-inch pots in a roofless, screened insectary. The portion of the leaf enclosed by the cell was drawn in a notebook, and daily records of oviposition, hatching, etc., were kept.

Weather records were obtained from maximum and minimum thermometers and a hygrothermograph in an instrument shelter nearby. Cutright,[8] and others as well, have recognized the limitations in the use of the mean of the daily maximum and minimum temperatures for studying biological phenomena. His method of calculating

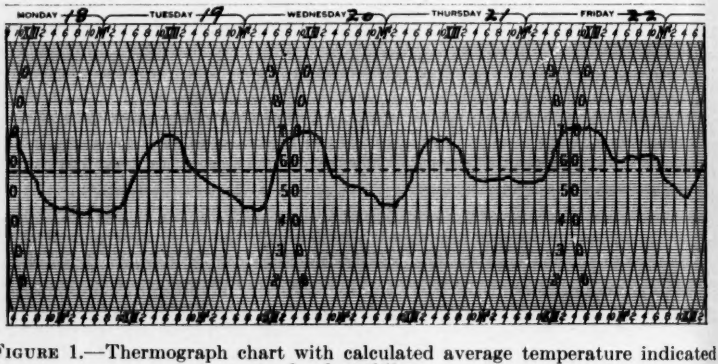

FIGURE 1.—Thermograph chart with calculated average temperature indicated by dotted line.

mean temperatures from planimeter measurements of thermograph charts was simplified somewhat by the use of the following formula:

$$T=\frac{RA}{A'}+t, \text{ where}$$

T = the average temperature,
R = the temperature range of the thermograph chart,
A = the area in square centimeters under the temperature curve for the period of observation (measured with a planimeter),
A' = the area of the chart in square centimeters for the period of observation (calculated from the dimensions of the chart),
t = the minimum recordable temperature on the chart.

The average temperature obtained from a mean of the temperature values at 2-hour intervals on the chart in figure 1 was 56.61. The average temperature computed by the above formula was 56.48. Theoretically, the formula gives the temperature value which would be obtained by taking the mean of an infinite number of observations.

In actual practice the temperature area was obtained from identical charts, and every period of development was recorded as "average

[7] NEWCOMER, E. J., and YOTHERS, M. A. BIOLOGY OF THE EUROPEAN RED MITE IN THE PACIFIC NORTHWEST. U. S. Dept. Agr. Tech. Bul. 89, 70 pp, illus. 1929.

[8] CUTRIGHT, C. R. NOTES ON THE COMPUTING OF MEAN TEMPERATURES FOR BIOLOGICAL USE. Ent. Soc. Amer. Ann. 20: 255-261, illus. 1927.

daily area." The conversion of area to average temperature is then reduced to

$$T=\frac{90\times A}{7.6\times 4.0}+10$$

$$=2.96A+10.$$

The temperature values which were plotted on cross-section paper were the average temperatures corresponding to individuals with identical developmental periods. For example, records were obtained on 12 individuals which developed from egg to adult in 15 days. Therefore, the average temperature for the 12 developmental periods was plotted against 15 days. The constants of equations and the curves representing the relation between average temperature and the developmental period were calculated by the method of least squares.

RESULTS

THE EFFECT OF TEMPERATURE AND SEASON ON THE INCUBATION PERIOD

The incubation periods of 1,895 eggs were observed over a period of 8 years. When the incubation period was plotted against the average temperature on semilogarithmic paper, the points fell approximately along a straight line, indicating that the exponential equation $y=ae^{-bx}$ would express the relationship (fig. 2). In this equation y is the incubation period in days, x is the average temperature in degrees Fahrenheit, and a and b are constants. The value found for a was 1,125.8, and for b, 0.073. Hence the relationship between the incubation period and average temperature is expressed by the equation $y=1{,}125.8e^{-0.073x}$.

This equation is used for estimating the incubation period. Suppose the area under a temperature curve for an estimated incubation period of a week was measured and the average temperature was found to be 64.3° F. Substituting 64.3 for x, the equation becomes $y=1{,}125.8e^{-4.69}$. From an exponential table it is found that $e^{-4.69}=0.0092$. Then $y=1{,}125.8\times 0.0092$, or 10.3. The observed value was 10 days.

When the average incubation period was plotted against the time in months from January to December, a U-shaped curve resulted (fig. 3). The incubation period averaged 20.4 days for eggs deposited in January. During May, June, July, August, and September the average ranged from 5 to 6 days. Eggs deposited in December required an average of 23.4 days to hatch, indicating that they were exposed to more cold weather subsequent to deposition than those deposited in any other month.

THE EFFECT OF TEMPERATURE AND SEASON ON DEVELOPMENT FROM EGG TO ADULT

A study of the data from 56 males and 176 females which were observed from egg to adult revealed that the relationship between the time of development and average temperature was also exponential. Data on males and females were examined separately but no significant difference in their rates of development was found. The equation and curve were developed from the combined data on males and females (fig. 4). The equation which expresses the relationship be-

tween average temperature and the time required for development from egg to adult is $y=739.35e^{-0.0564x}$, where x is the average temperature and y the period of development.

Only 10 or 12 days are required for development from egg to adult in the summer. The periods are much longer in the winter months

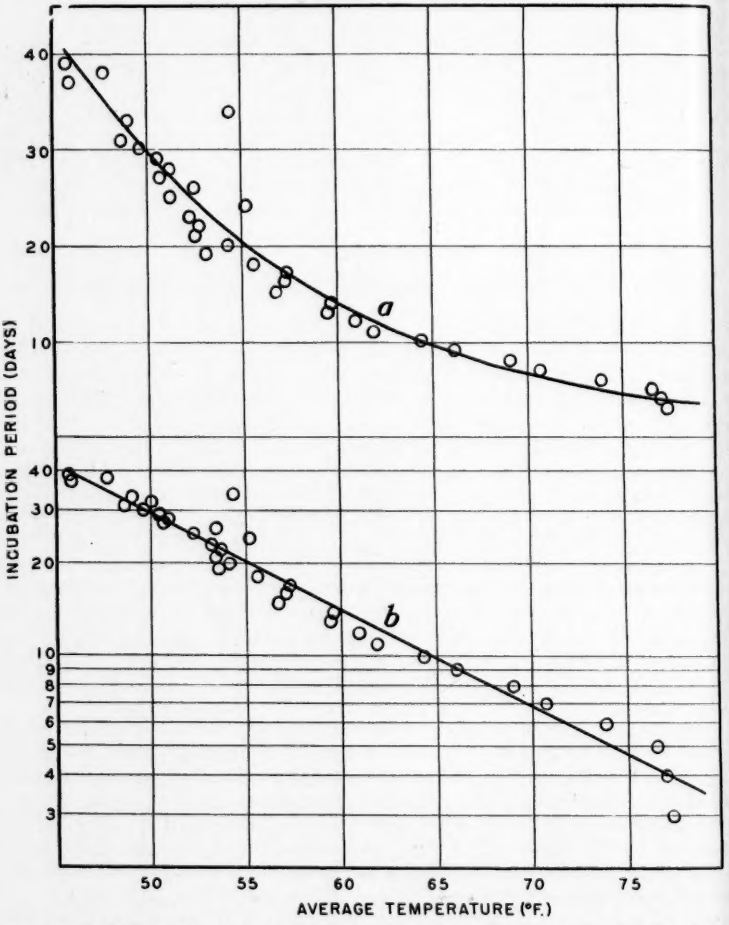

FIGURE 2.—The relationship between average temperature and the length of the incubation period: *a*, Data plotted on cross-section paper; *b*, data plotted on semilogarithmic paper.

and an extreme of 50 days was recorded for 1 female. The average length of these developmental periods forms a U-shaped curve as the season proceeds from January to December (fig. 5). An average of 34.5 days was required for egg to adult development from eggs deposited in January. In February this average dropped to 26.2, and

by March to 19.9 days. Broods beginning in May, June, July, and August required 12 days or less for development, but by November the requirement had advanced to more than 30 days.

DEVELOPMENT FROM EGG TO EGG

After becoming adult, females may spend from 3 to 20 days, or even more, before depositing the first egg. The time spent in the period of preoviposition varies greatly with the season, just as other periods of development vary. The life cycle ranged from 13 to 15 days in the summer to 61 days for a brood beginning in December.

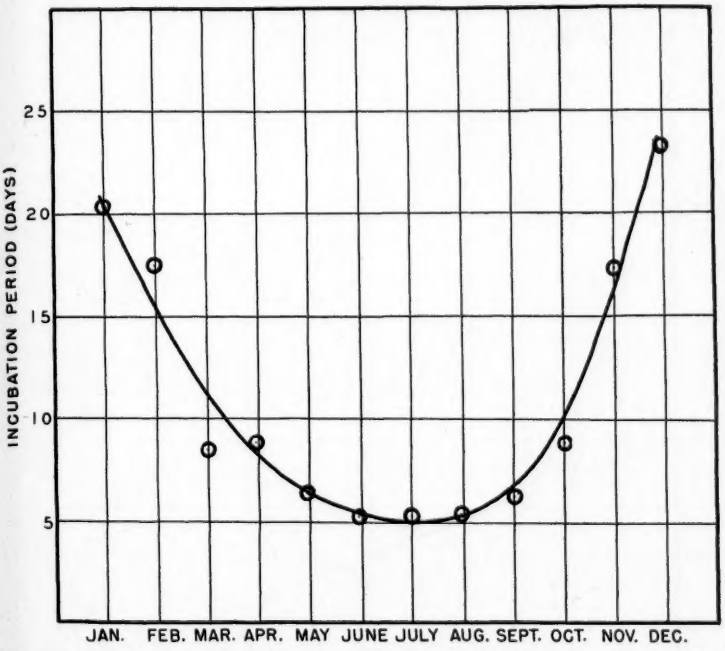

FIGURE 3.—The relationship between the season and the average length of the incubation period.

A study of the length of the life cycle of females and the average temperature disclosed that the data approximated a straight line when plotted on semilogarithmic paper (fig. 6). The equation derived from these data was $y=1{,}305.6e^{-0.0607x}$, where y is the time in days from egg to egg and x is the average temperature.

THE ADULT LIFE SPAN

The outstanding difference in the life history of male and female mites is the length of life after reaching the adult stage. Adult males originating in June, July, and August, lived only an average of 5 or 6 days, while females lived about 13 days in the adult stage. The average adult life span of 126 females was 21.8 days and that of 55 males was 14.5 days. The seasonal differences are shown in figure 7.

The influence of temperature on the adult life span was not so marked as on development, but it was evident that a general correlation existed. A straight line, rather than an exponential curve, appears to be a valid expression of the relationship between average

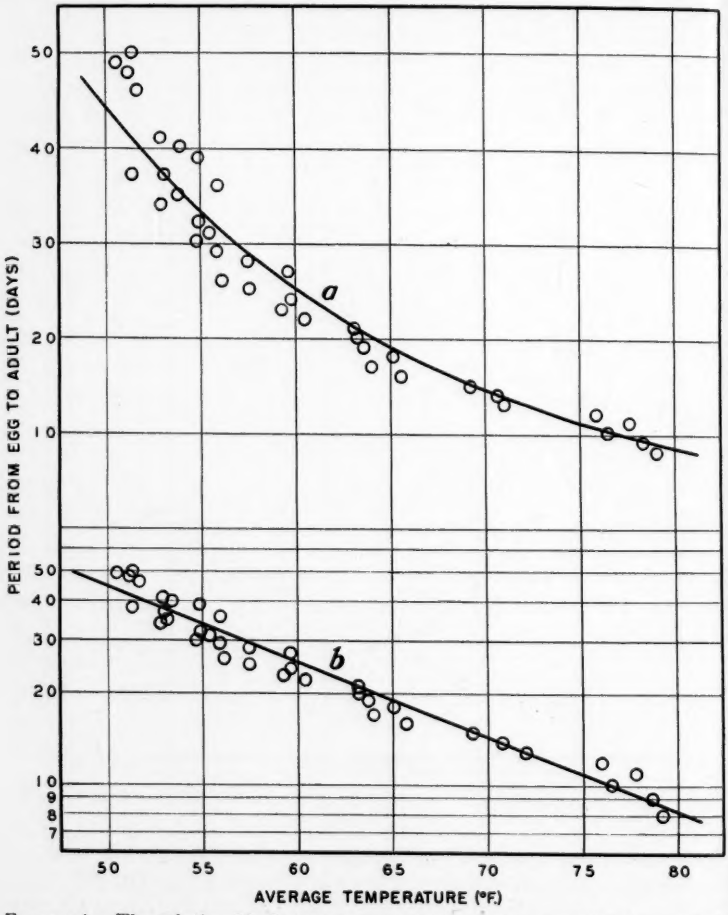

FIGURE 4.—The relationship between average temperature and development for the period from egg to adult: *a*, data plotted on cross-section paper; *b*, data plotted on semilogarithmic paper.

temperature and the adult life span (fig. 8). The line fitted to the data on males has the equation $y=-1.13x+94.44$, and that for females has the equation $y=-1.40x+117.25$, where y is the adult life span in days and x is the average temperature.

THE TOTAL LIFE SPAN

Studies were made of the average temperatures of birth-to-death periods of both males and females but no satisfactory relationship was established. Since the time of development from egg to adult

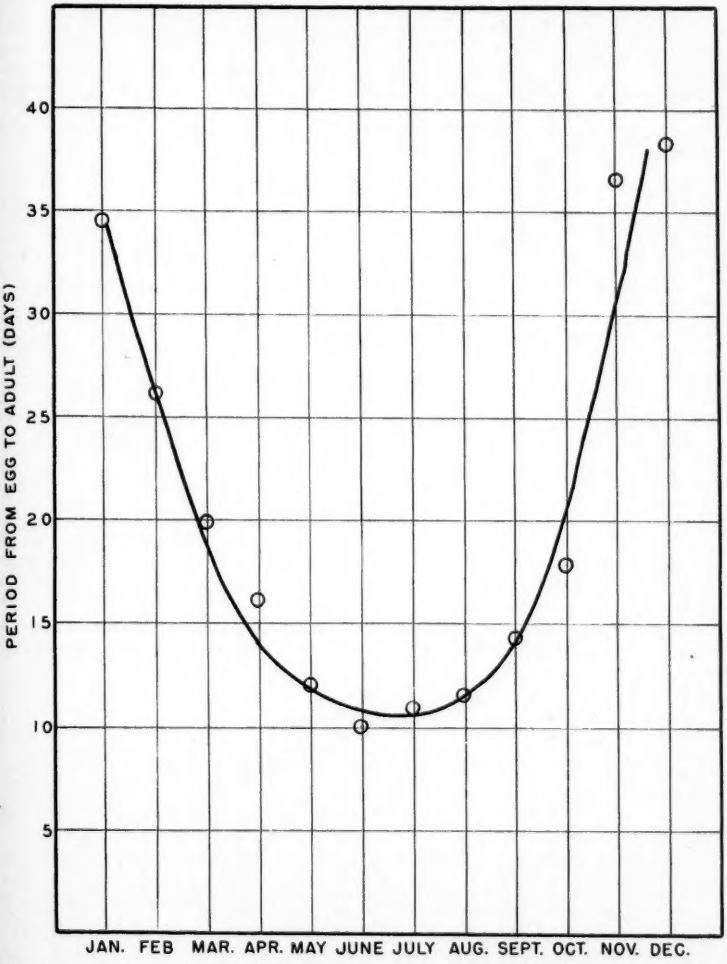

FIGURE 5.—The relationship between season and development for the period from egg to adult.

was found to be an exponential function of average temperature, while the adult life span was a linear function, it is evident that, although there is a relationship between the birth-to-death period and average temperature, it cannot be expressed by a simple equation.

The difference in the total life span of the sexes arises mainly from the difference in the longevity of the adults. The average total life span of females varied from 39.3 days for those originating in January to 16.6 in July and 60.3 in November. Males averaged 34.0 days for

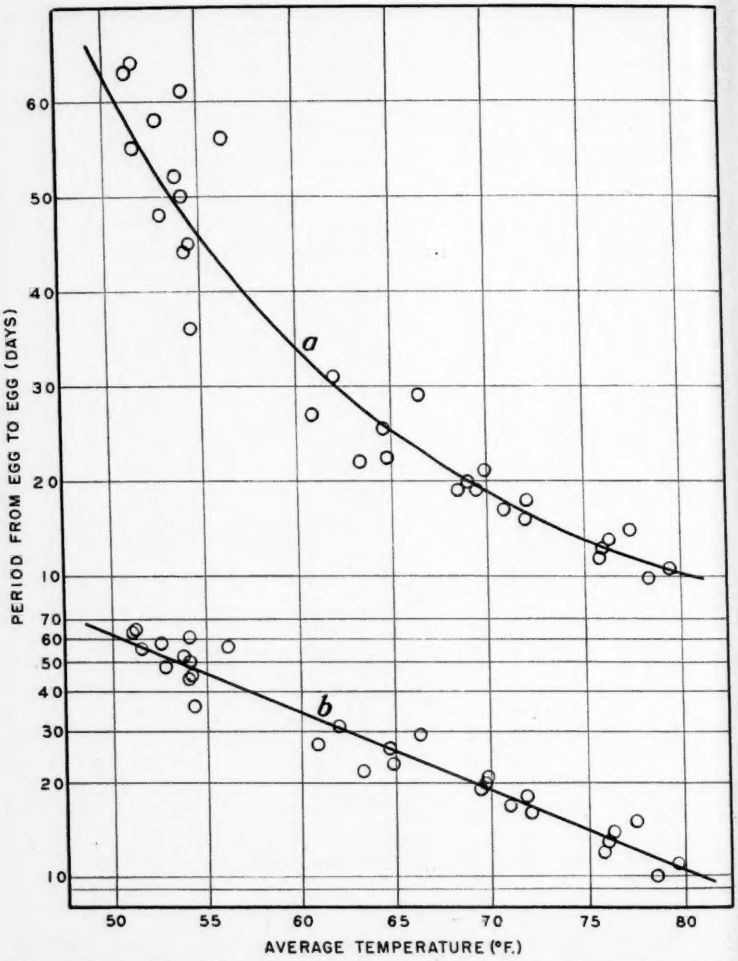

FIGURE 6.—The relationship between average temperature and development for the period from egg to egg: *a*, Data plotted on cross section paper; *b*, data plotted on semilogarithmic paper.

January and declined to a minimum of 10.2 days for June, but one male which originated in November lived for 94 days (fig. 9). The average length of life for 125 females over all seasons was 30.4 days; the average for 55 males was 22.9 days.

THE RELATIONSHIP BETWEEN OVIPOSITION AND THE ADULT LIFE SPAN OF FEMALES

The curve representing the adult life span of females originating in the months of January to July, inclusive, was very similar to the curve representing the number of eggs deposited (fig. 10). The average adult life declined from 38.0 to 13.2 days for this period, and the average number of eggs deposited ranged from 39.0 to 15.8 per female. It will be observed that the average number of eggs laid exceeded the average number of days spent in adult life. Hence, more than 1 egg per day was deposited. The maximum rate was 1.50

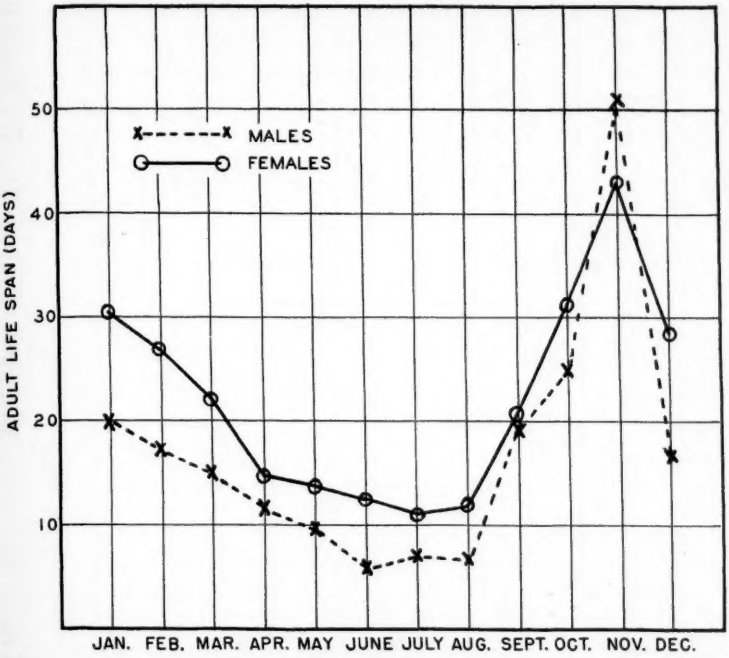

FIGURE 7.—A comparison of the adult life span of males and females in different seasons.

per day by females originating in February. Beginning with August, the egg-curve dropped below the life-span curve and remained below it the rest of the calendar year. Oviposition reached the minimum rate of 0.21 eggs per day by females originating in October. The data used are for the year 1938 and the value plotted for December is probably in error, since only one individual originated in this month.

THE VARIATION OF MITE POPULATION WITH THE SEASON

From monthly egg counts on 10-cm.^{2} disks punched from untreated leaves an indication of the variation of mite population with the season was obtained. A curve was prepared from the average of

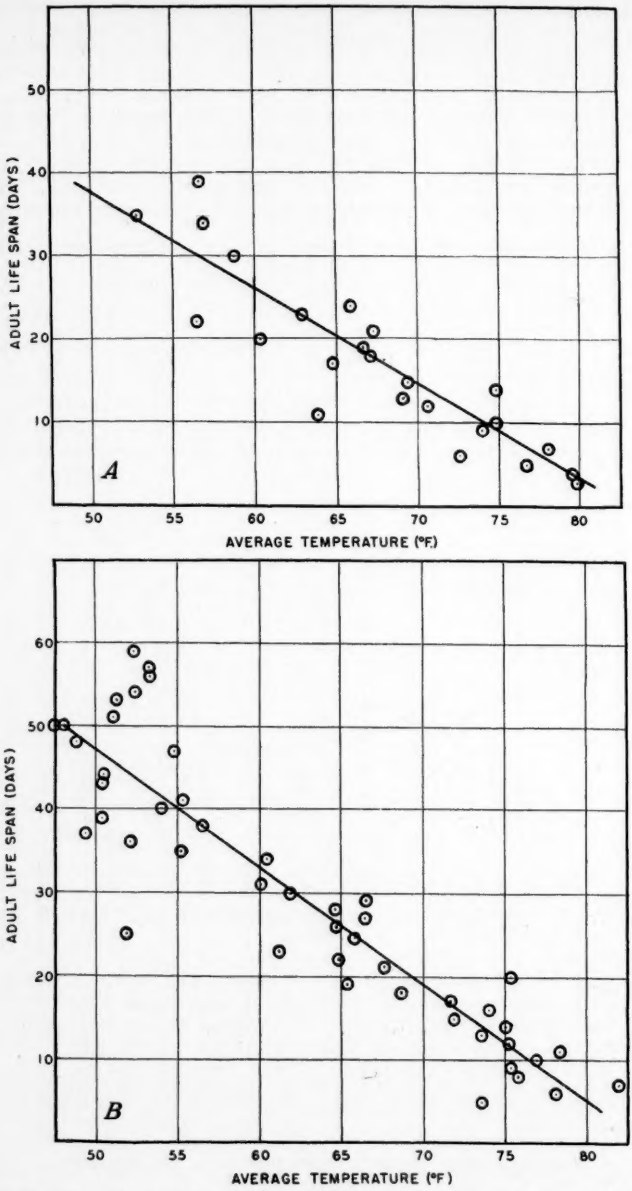

FIGURE 8.—The relationship between average temperature and the adult life span of males (*A*) and females (*B*).

counts made during 6 years (fig. 11). Ordinarily the population of mites is highest during March, April, and May. It will be observed that there was a gradual decline in the number of eggs as warm weather approached. The peak indicated at the last of June was obtained from an unusually heavy infestation which developed 1 year out of the 6. It is believed that this was exceptional. During the hot months of July, August, and September, egg counts were very

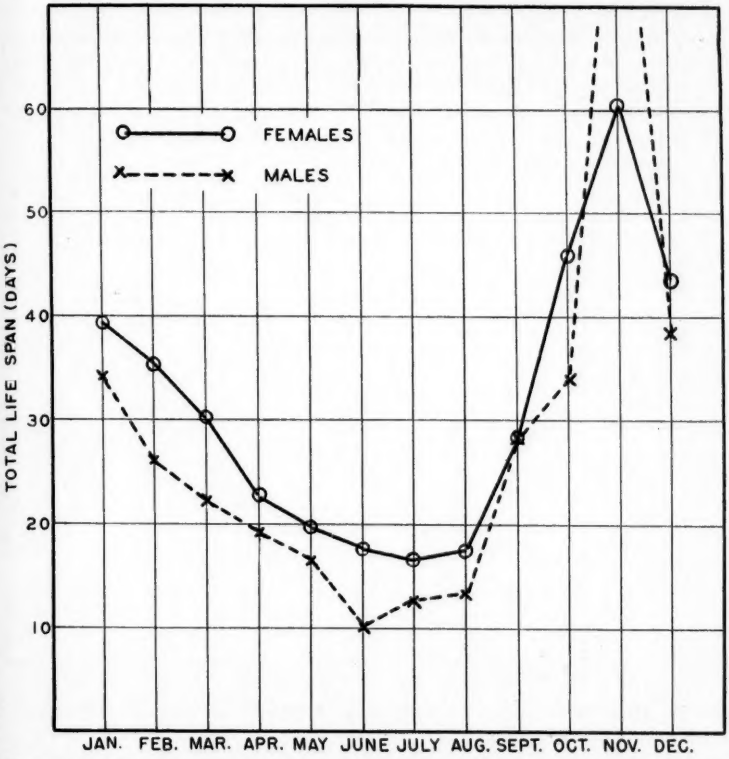

FIGURE 9.—The relationship between the season and the total life span (birth to death) of males and females.

low, indicating unfavorable conditions for mites. With the advent of cool weather in the fall the population began to build up again.

Inasmuch as the development of the citrus red mite has been shown to be a function of temperature, it may be asked why the population does not reach its greatest density in the summer. Although the time required for the incubation of eggs and development is shortest in the summer, other factors are of more importance. For example, at 50° F. the calculated time for development from egg to adult is 44.1 days; at 80° the time is 8.1 days. Hence, development at 80° is 5.4 times as fast as at 50°. But the adult life span is more than 9 times as long at 50° as at 80° (fig. 8). Thus the rate of development in hot

weather is more than offset by the longer life in cool weather. This factor alone would account for an increase in the population in cool weather. To increase further the population potential for cool weather is the fact that during the season of greatest population density more eggs per female are deposited (fig. 10). In addition, high temperatures are probably lethal to the mites, since if the curves in figure 8 were extrapolated the thermal death-point would fall at about an average temperature of 85°.

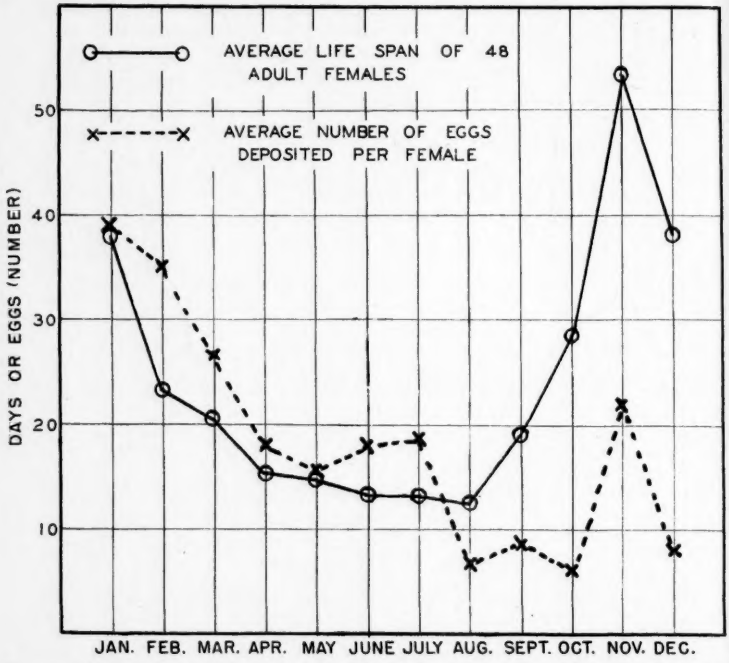

FIGURE 10.—The relationship between the season, the average life span of adult females, and the average number of eggs deposited.

In spite of favorable temperature conditions in the fall, mite populations do not usually become dense at this season of the year. In the first place, the population must recover the momentum lost in the summer. In the second place, the rate of egg production per female is low. It is possible, too, that the quality of the food is an important influence on mite population. In the fall of the year trees have a load of ripening fruit and at the same time are entering a semidormant stage. It will be noted that the population becomes dense in the spring when fertilizing and cultural practices usually have been started in the orchards and the trees assume vigor. These factors, in addition to temperature conditions, are probably favorable to the mite.

SUMMARY

Several phases of the life history of the citrus red mite were found to be mathematically related to the average temperature computed from thermograph charts. The length of the incubation period, the time for development from egg to adult, and the time for development from egg to egg were found to be functions of average temperature, which could be approximated by the exponential equation $y=ae^{-bx}$, where y is the period of time in days and x the average temperature in degrees Fahrenheit. The duration of adult life was found to be a linear function of average temperature, conforming to the equation $y=-ax+b$, where y is the time in days and x is the average temperature.

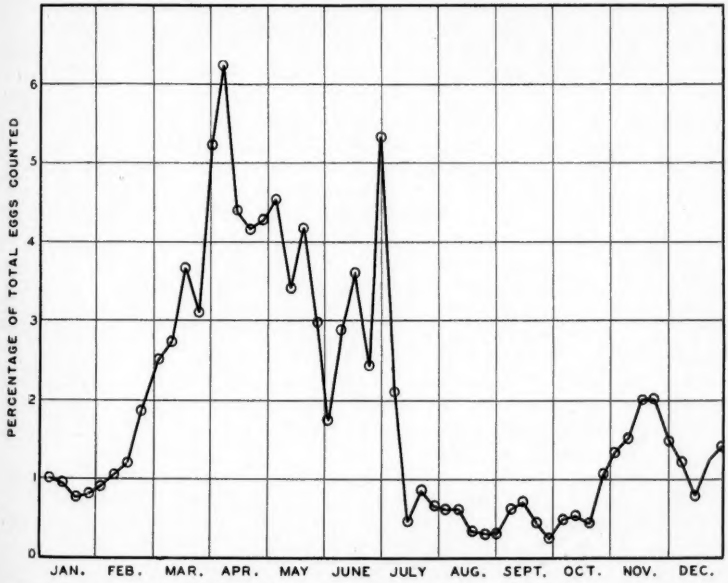

FIGURE 11.—The seasonal density of citrus red mite as indicated by egg counts.

The average incubation period and the time for development from egg to adult formed U-shaped curves when plotted against the months from January to December. The adult life span and the total life span of mites also approximated U-shaped curves when plotted against the months of the year. The adult life span of males was found to be shorter than that of females.

The population of the citrus red mite declines in hot weather and increases in cool weather. The early spring months apparently afford optimum conditions. Although the temperatures at this season do not induce the most rapid development, other factors, such as the long life of the adults and the high rate of egg production, aid in promoting the maximum population.

CYCLOCEPHALA (OCHROSIDIA) BOREALIS IN CONNECTICUT[1]

By J. PETER JOHNSON

Assistant entomologist, Connecticut Agricultural Experiment Station

INTRODUCTION

Since grubs of annual species of scarabaeidae have become more prevalent and injurious to turf in the northeastern part of the United States during the last two decades, it is increasingly important that the biology and description of such pests be made available to economic entomologists.

Cyclocephala borealis Arrow [2] has recently become a serious pest of lawns in Connecticut. In order to determine how to combat it successfully, the studies described below have been carried on over the past 3 years.

Cyclocephala borealis occurs as far south as Alabama and westward to California,[3] its range extending over most of the United States. It was found in Connecticut for the first time when grubs collected from injured turf on an estate in Westport (*4*),[4] November 12, 1931, were identified as larvae of this scarabaeid.

During October 1936, two different lots of grubs were received for identification from Greenwich, where severe lawn damage had occurred. In the fall of 1937, grubs were received from East Norwalk, Fairfield, and Greenwich. Upon investigation it was found that approximately 15 acres of lawn area had been badly injured or destroyed by the insect. In 1938, additional infestations were found in Greenwich and Norwalk, and another was reported from East Hartford. Other infestations were found in 1939 on two golf courses in Fairfield and on one course in Stamford. It is evident from the information at hand that a scattered infestation exists in the shore towns in southwestern Connecticut.

This insect is usually a pest of grassland, and a brief account of its injuriousness to lawns in Ohio, together with certain experiments in control methods, has been given by Neiswander (*8*). It has also been reported by Swenk (*11*) as injurious to winter wheat in Nebraska.

Certain morphological and biological studies of *Cyclocephala* (*Ochrosidia*) *borealis* have been made in recent years. Saylor (*9*) has described the adult male and published illustrations of the male genitalia. Sim (*10*) has described the larva and illustrated the epipharynx and raster. Neiswander (*8*) also reported on the life history of this insect. The biology of *C.* (*villosa*) *borealis* as reported by Hayes (*6*) in 1918 was really that of *C. immaculata* Oliv. (*7, p. 67*).

[1] Received for publication June 15, 1940.

[2] Dr. E. A. Chapin, U. S. National Museum, confirmed the identification of this scarabaeid as *C. borealis* Arrow from duplicate material submitted to him.

[3] U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE. THE INSECT PEST SURVEY BULLETIN. 1930, 1931, 1934, 1937, 1938. [Mimeographed.]

[4] Italic numbers in parentheses refer to Literature Cited, p. 86.

SYSTEMATIC POSITION

This scarabaeid was originally described by Burmeister, under the subfamily Dynastinae, tribe Cyclocephalini, as *Cyclocephala villosa*. Since two species received the name of *Cyclocephala villosa*, the first being Blanchard's Bolivian species and having priority, Arrow (*1, p. 172*) renamed the North American species *borealis*. Casey (*5, pp. 109–147*), in his review of the subfamily Dynastinae, placed *villosa* in the genus *Ochrosidia*. Arrow considers *Ochrosidia* as a subgenus. The Leng Catalogue (*2, p. 54*) lists the species as *Cyclocephala borealis* Arrow.

DESCRIPTION AND LIFE HISTORY

THE EGG

The eggs of *Cyclocephala borealis* when laid are pearly white, ovoid, and delicately reticulate. The sides of some are nearly parallel, with rounded ends. When first laid, 15 eggs had a mean length of 1.68 ± 0.02 mm. and a mean breadth of 1.3 ± 0.01 mm. The eggs begin to swell when 2 or 3 days old, reaching a maximum size just before hatching. Eight other eggs measured 1 day before hatching had a mean length of 1.68 ± 0.03 mm. and a mean breadth of 1.58 ± 0.04 mm. It will be noted that the mean length of the newly laid eggs is the same as that of those ready to hatch, 1.68 mm. This would indicate that the egg enlarges transversely during the development of the embryo.

While eggs have been collected in the field as early as June 29, deposition continues throughout the flight period. The majority are laid in turf between 4½ and 6 inches below the surface. Some have been found at a depth of only 1½ inches but none below a depth of 6 inches. The maximum number of eggs laid by a single female beetle under insectary conditions was 29 and the minimum 3. A total of 362 eggs was laid by 32 females, the average being between 11 and 12 per female. Females, collected in the field from pupal skins on July 6 and placed with males on July 7, deposited eggs on July 8. The majority of the eggs in the insectary hatched in 20 to 22 days. Just before the larva hatches, the mandibles and portions of the head capsule, which have become darkened in color, are visible through the chorion.

THE LARVA

The larva passes through three instars before attaining full growth. The mean dorsal length of twenty-one 1-day-old larvae was 5.08 ± 0.05 mm. The mean cranial width of 20 first-instar larvae was 1.57 ± 0.01 mm. The mean cranial width of 15 second-instar larvae was 2.32 ± 0.01 mm. Full grown third-instar larvae differ somewhat in size. The mean length of 27 specimens was 22.7 ± 0.12 mm., individuals ranging from 20 to 24 mm. The mean cranial width of 44 specimens was 4.12 ± 0.01 mm.

The head of the full-grown larva is yellowish brown in color, and the body is white. The anal slit is transverse and arcuate. The raster has a sparse group of coarse, long, hooked, brownish spines, which become larger toward the anal slit. Figures of the full-grown larva, together with structural characteristics of the epipharynx, mandibles, maxillae, and prothoracic and metathoracic legs are given in figure 1.[5]

[5] The figures of the pupae and the epipharynx of *C. immaculata* were prepared by the author; all others are by Elizabeth Kaston.

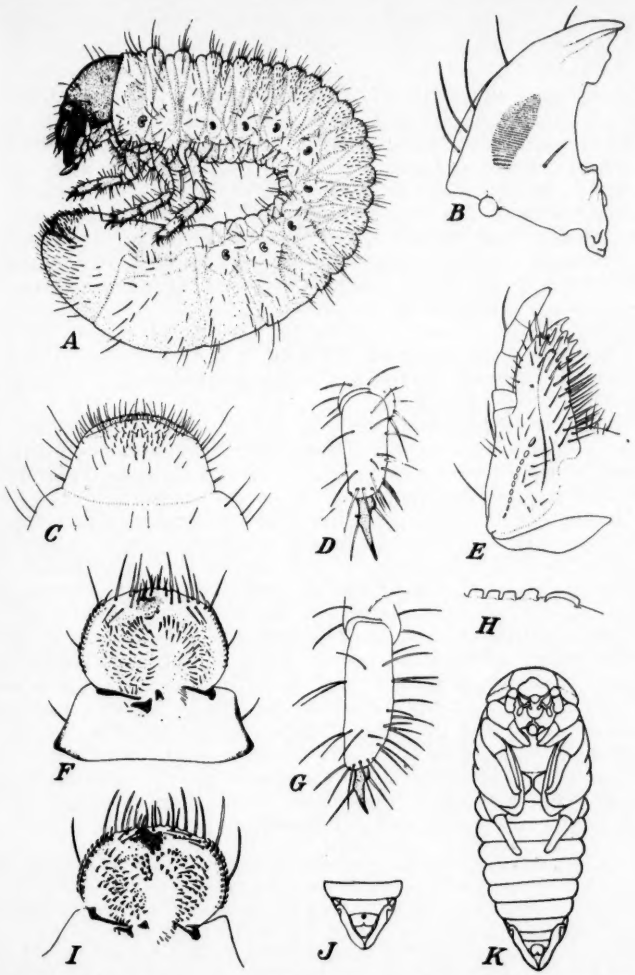

FIGURE 1.—Larva and pupa of *Cyclocephala borealis:* *A*, Larva entire; *B*, right mandible of the larva, showing area of striation; *C*, ventral aspect of the anal segment of the larva; *D*, prothoracic leg of the larva; *E*, cephalic aspect of the left maxilla of the larva, showing one very large, seven median, and two small stridulating teeth; *F*, epipharynx of the larva; *G*, metathoracic leg of the larva; *H*, the five anterior stridulating teeth of the left maxilla of the larva; *I*, epipharynx of *C. immaculata*; *J*, ventral view of the last three segments of the female pupa; *K*, ventral view of the male pupa.

Morphologically the larvae of *Cyclocephala borealis* Arrow and *C. immaculata* Oliv.[6] are very similar, but the haptomeri of the two

[6] Appreciation is hereby acknowledged to Dr. P. O. Ritcher for the receipt of many fine larval specimens of *C. immaculata* from Kentucky.

species are somewhat different in structure. A figure of the epipharynx of *immaculata* is shown in figure 1, *K*. The haptomerum of *borealis* has a definite single, large, sclerotized, rounded plate and straight apex, with a deep notch to the left of the center of the zygum. The darkened chitinized anterior edge on the right lobe is about four times as broad as it is deep. The breadth of the left lobe likewise is about twice its depth. In *immaculata* the rounded, sclerotized plate does not terminate as definitely as that of *borealis*, and the notch is more centrally located but rather indistinct. The lobes are more nearly equal in size, with the dark, chitinized apical edge of the right lobe more than two times as broad as it is deep, while the breadth and depth of the left lobe are about equal. The dark, chitinized apices of the lobes are more distinct than in *borealis*.

Feeding begins almost immediately after hatching, and larvae less than 1 day old are discolored by food in the alimentary tract. The larvae feed on organic matter and on the roots of plants, in Connecticut primarily on the roots of turf.

Damage to turf usually occurs in September or October and in the spring. Three diggings in different localities where such injury was evident yielded, respectively, 33, 48, and 64 third-instar larvae to 1 square foot. Third-instar larvae were observed in the field on September 7, and it is evident from field observations that the larvae are in this instar when they go into hibernation.

The larvae react to changes in soil temperature in a manner similar to that of other scarabaeid larvae, descending at the onset of cold weather in the fall and ascending in the spring. Larvae in individual cases will descend to a depth of 18 inches to hibernate. In 1939 the descent began early in November, when the larvae were found distributed at depths ranging from 2 to 14 inches, as shown in table 1. One digging made on April 12, 1938, before the upward movement of the grubs took place, revealed 11 larvae in the upper 3 inches of soil, 19 larvae at a depth of from 3 to 9 inches, and 1 larva more than 9 inches deep. Winter mortality may be heavy; in one digging 30 of 61 grubs found were dead. At a later date in diggings made elsewhere, many dead grubs were noted at hibernation depths.

TABLE 1.—*Depth to which larvae of Cyclocephala borealis descend to hibernate, 1939*

Date	Number of larvae found at depth indicated (inches)												
	2	3	4	5	6	7	8	9	10	11	12	13	14
Nov. 9	6	5	0	4	3	4	4	2	2	2	1	0	0
Nov. 14	0	2	4	4	9	2	6	3	5	0	2	0	1

THE PUPA

The pupa when newly transformed is creamy white in color, but it gradually turns a reddish brown. The mean dorsal length of 16 preserved pupae was 16.78 ± 0.18 mm. Figure 2 illustrates the differences between the sexes. The male genital segment (ninth abdominal) bears a pair of conspicuous lobes. In the female this segment is partially divided by a short median suture, anterior to which is situated the gonotreme. The sex of the older pupae may also be determined by observing the sexual differences of the tibial spurs through the cuticle of the prothoracic legs.

The pupal period occurs during June. On June 8, 1939, a digging adjacent to the shore of Long Island Sound yielded 25 larvae, 51 prepupae, and 41 pupae. A second digging was made at the same place on June 19, 1939, and 3 larvae, 2 prepupae, and 36 pupae were

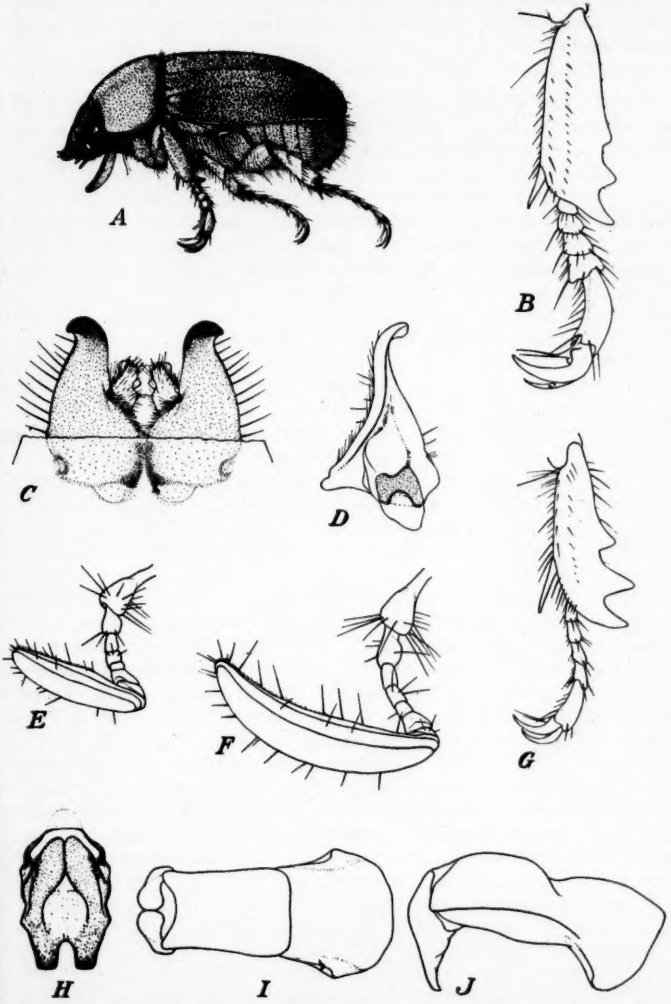

FIGURE 2.—*Cyclocephala borealis: A*, Adult male; *B*, prothoracic leg of the adult male; *C*, dorsal aspect of the mandibles of the adult male in situ; *D*, mesal aspect of the right mandible of the adult; *E*, female antenna; *F*, male antenna on same scale as *E*; *G*, prothoracic leg of the adult female on same scale as *B*; *H*, end view of the male genitalia; *I*, dorsal view of the male genitalia; *J*, lateral view of the male genitalia.

found. An examination of larvae placed in a 24-inch glazed tile the preceding fall was made on June 9, 1939, and 42 larvae and 2 prepupae were found. About 75 larvae were used for rearing purposes at room temperature and also at 78° F. The prepupal period was 4 to 5 days in length. The length of the pupal period varied from 11 to 16 days, but the largest number emerged as adults on the fourteenth and fifteenth days.

THE ADULT

The adult is chestnut brown in color and covered with fine hairs, the male being slightly larger and somewhat darker than the female. The mean dorsal length of 22 adult males collected from pupal skins was 11.84 ± 0.10 mm. and the breadth 6.76 ± 0.06 mm. The mean dorsal length of 22 females collected from pupal skins was 11.03 ± 0.07 mm., and the breadth 6.66 ± 0.04 mm. The males are distinguished by their larger tarsal claws, shorter and narrower tibial spurs, and longer antennal lamellae. In figure 2 are drawings of a male, the prothoracic legs and antennae of the male and female, and the male genitalia. The body of *Cyclocephala borealis*, (*3, pp. 989–990*), is pubescent above, and the antennal club of the male is longer than all the other antennal joints together. *C. immaculata* is smooth above, and the antennal club of the male is shorter than all the other antennal joints together.

The adults are nocturnal in habit and remain in the soil during the day. Careful observation during the evening when the adults were active and again during daylight hours failed to indicate feeding of any kind. The alimentary tract was empty and atrophied in all specimens examined. The mandibles are fully developed but apparently unfitted for chewing, for they are anteriorly excurvate rather than incurvate (fig. 1, *C*).

Just about dusk, or half an hour before dark, on warm, humid evenings, adults may be observed emerging from the soil and climbing to the tips of the grass in lawns. Occasionally one will take wing and fly rapidly just above the ground. As the darkness deepens, more and more beetles emerge and greater activity is observed. From investigations made during the last 2 years when adults were present, it appears that males are predominant in activity and flight. They usually fly from 1 to 2 feet above the ground in search of females. Some, however, will fly somewhat higher and dart away in the dark or toward a light to which they are attracted. When the insect takes to flight, the wing vibrations have a characteristic sound. Flight and general activity cease before midnight. On cool evenings, flight is lessened and the adults are present only for a brief period.

The female beetles emerge and rest on the surface of the soil, if it is void of grass, or in the turf areas ascend to the tips of the grass. In a moment they are captured by a male, and copulation takes place directly. The males clasp the females along the edge of the elytra behind the legs with their tarsal claws. The females often move about, carrying the males. If disturbed, when single or paired, the adults invariably immediately seek shelter in the grass or loose soil.

In the rearing experiments, adults were removed directly from the soil in their pupal cases, they mated the next day, and eggs were obtained the third day. When individuals were placed together upon collection from their pupal cases in the field, mating immediately took place, and the following day eggs were obtained.

In 1938 the first adults were captured around the lights and windows of a private garage on the evening of June 24. The largest number were captured about July 5, and the last on July 25.

The life cycle requires 1 year; and the stages, briefly summarized, are as follows: Egg, 20 to 22 days in June and July; larva, 10 to 11 months from July to June; prepupa, 4 to 5 days in June; pupa, 11 to 16 days; and adult, 5 to 25 days in June and July. As the transformations do not all occur at the same time, there is overlapping of the stages.

CONTROL

The larvae are to some extent parasitized by a species of *Tiphia* as yet undetermined. Of 1,000 larvae examined, 25 were found to be so parasitized.

Applications of lead arsenate, made in 1938 under the author's direction in Westport and Greenwich at the rate of 20 and 30 pounds, respectively, to 1,000 square feet of lawn, gave excellent control. Neiswander (*8*) reports that he obtained approximately 70 percent control in 1 month, using lead arsenate at the rate of 10 pounds to 1,000 square feet. He also reported good control by applications of carbon bisulfide emulsion diluted with water.

Light traps were used by the author in Westport to capture adult beetles. At first the traps were suspended on standards 3 to 4 feet above the ground, but after the flight habits of the beetles were observed, the traps were set directly on the ground and an increased number were captured. Homemade traps, consisting of an ice-box drip pan containing water with a layer of kerosene oil one-fourth of an inch in depth, four pieces of window glass 9 by 12 inches placed vertically to serve as baffles, and a 100- or 150-watt frosted bulb suspended directly above the baffles, were very successful. A 300-watt daylight bulb was tried in the area of immediate infestation but was not so successful as the lower-powered bulbs.

Three such traps were used in one area of about 3 acres and a total of 18,967 beetles were captured. Of 9,029 adults captured in two of the traps and determined for sex, 8,996, or 99.63 percent, were males and 33, or 0.37 percent, were females. The traps were in operation from June 25 to July 29. Thirteen of the females were captured on the night of July 5 when the maximum catch was made, and the remaining 20 were taken on 6 other nights. This result was similar to that obtained by Neiswander (*8*). Light traps are not recommended for control. However, if the beetles should be attracted by lights to residences or recreation centers and become a nuisance, light traps might be useful.

SUMMARY

Cyclocephala borealis Arrow was first found in Connecticut in 1931, in the town of Westport, where larvae were destroying lawn turf. It has since been found in several other towns in the southwestern part of the State, and during the past 9 years has injured or destroyed many acres of fine turf on lawns and golf courses.

The adults are chestnut brown in color and are present for about 1 month, beginning about June 25. They are nocturnal in habit, emerging from the soil about dusk, and are active during the early hours of the evening. The males are the more active.

Oviposition begins the first or second day after the adults first emerge, and the eggs hatch in about 3 weeks. The larvae pass through three instars, usually entering the third instar in early September. A field population of 64 larvae per square foot was recorded in one lawn. The larvae feed upon the roots of grass, and turf injury may first appear in September. The larvae ascend and descend in the soil with the rise and fall of the soil temperatures in the spring and fall. Hibernation occurs in the larval stage during the winter at depths varying from 2 to 18 inches, with the majority of the larvae at depths between 3 and 9 inches. The larvae complete their growth by late May or early June. The insect spends from 4 to 5 days as a prepupa in early June. It remains in the pupal stage some 2 weeks, emerging as an adult about the third week in June. The adults have not been observed to feed, and their mouth parts are unsuited for feeding.

Both male and female beetles are attracted to lights. However, light traps capture a great predominance of males and are not recommended for control. The larvae may be controlled by treating the turf with lead arsenate or carbon bisulfide emulsion diluted with water.

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GERMINATION REDUCTION AND RADICLE DECAY OF CONIFERS CAUSED BY CERTAIN FUNGI[1]

By PAUL LEWIS FISHER

Collaborator, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Increased attention has recently been directed to the frequent recurrence of failures of emergence in coniferous nursery beds. Sometimes the failures have been severe, completely covering large blocks of beds; at other times they have been spotty over several beds. It has long been known from observation (*2, 3, 4*)[2] that decreases in emergence of pine seedlings may be indicative of damping-off epidemics, and it has been shown by inoculations with damping-off organisms that emergence failures can be produced in steamed soil and that failures due to these organisms can be controlled in the nurseries by the same soil-disinfectant treatments that control damping-off.

There is reason to suspect, however, that seeds are decayed by organisms other than those known to cause damping-off. Some rather serious failures have occurred in beds sown with seed from lots that had germinated well in other trials. Numerous failures have been observed where soil cover and moisture were apparently correct and damping-off fungi were not appreciably active. Failures have been most frequent in cold, wet soil where such emergence as did occur was slow, and nonsprouted seeds removed from such beds have often been found to be rotted.

In routine seed investigations a cutting test is made on each lot of seed collected. One hundred seeds are cracked open and classified, according to the appearance of the embryo, into good seed, rancid seed, and "blind" seed, that is, seeds that have not been properly filled. The cutting test is indicative of the germination potential of the lot. Frequently, however, the percentage of germination in the field is lower than the percentage of seed classified as good. Some of the seeds, therefore, that appear viable may be immature or in a weakened condition and thus may be more susceptible to attack by organisms that may or may not usually be parasitic. It is known that the micropyle of the bean is occasionally glutted with bacteria. While coniferous seeds have hard coats and no similar open chamber for the carrying of organisms, there is some opportunity for organisms to enter the seeds before they fully mature in the cone, as a result of injury in the extraction process or because of improper storage. If decay occurs in a treated soil, therefore, it is conceivable that the pathogen was carried into the soil in or on the seed itself.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 94.

PREVIOUS INVESTIGATIONS

Many investigations have been reported on the causes and control of damping-off in both deciduous and coniferous seedbeds. Active work on the control of damping-off of conifers is still in progress. The term "germination loss" has been used for reduced emergence resulting from seed decay or preemergence damping-off, or both, since in ordinary seedbed practice the seed is covered and the two types of loss cannot be distinguished. Hartley (*2*, *3*, *4*) attributed this loss in coniferous seedbeds to species of *Pythium*, *Rhizoctonia*, and *Fusarium*, on the basis of successful inoculations on steamed soil with representatives of all three genera, including *P. aphanidermatum* under the name of *Rheosporangium*. *Fusarium* spp. on the whole indicated less ability to cause preemergence than postemergence losses. For *Botrytis cinerea* in a preliminary test the reverse appeared true.

Rathbun-Gravatt (*5*), by inoculations under direct observation, demonstrated the ability of a number of strains of *Pythium*, *Rhizoctonia*, and *Fusarium* to reduce sprouting of coniferous seed. She found that, in general, the fungi that decreased sprouting were the ones that were able to decay radicles.

Fourteen species of fungi "arising from the seed of *Pinus sylvestris*" were determined by Garbowski (*1*). His methods of sterilization and isolation are not known, however. The germination of *Pinus sylvestris* was greatly reduced by contaminations of *Pyronema omphalodes* and one species of *Botrytis*.

Vanin (*8*) and Vanin and Kotchkin (*9*) studied the fungi associated with the seeds and seedlings of tree species. They reported inhibited germination from widespread contaminations of *Penicillium*, *Aspergillus*, *Mucor*, and *Rhizopus*. They grouped seed fungi into three classes: (1) Those that damage seeds; (2) those present in or on seeds but not causing diseases until after germination (damping-off); (3) those not harmful to either seed or seedling.

Ten Houten (*7*) isolated many fungi from conifer seeds or seedlings; part of the isolations from seed (which ones are not indicated) had been preceded by surface sterilization. Inoculations with more than 50 of these and some others were made on 24 surface-sterilized seeds each of Scotch and Austrian pine in tube cultures, half of which were on filter paper and half on sterilized soil. In his filter-paper cultures (and in the soil tubes also) he had the seed under direct observation, and germination reduction is to be taken more as an indication of seed decay than of preemergence damping-off. So far as inferences can be drawn from so few seeds, there was fairly definite reduction of sprouting by one of the two *Rhizoctonia* lines, by *Botrytis cinerea* and an unidentified *Botrytis*, and very likely some reduction by *Pythium debaryanum* (which the author is understood to regard as including *P. ultimum*), *Phytophthora fagi*, and *Coniothyrium pityophilum*. For the remaining fungi, apparent increases in germination associated with the inoculation were as frequent and as large as the decreases except for the presumably accidental case of *Pyronema confluens* on Scotch pine. Except for the *Pyronema*, all of these fungi that appeared to reduce germination also attacked seedlings, though the *Phytophthora* was relatively weak. There thus appeared, as in Rathbun-Gravatt's work, to be considerable correlation between ability to attack seed and to attack seedlings. However, a number of *Fusarium* species and

related fungi included in the test, and also other species of *Pythium* and another line of *Rhizoctonia*, attacked seedlings without any material reduction in germination.

Pinus pinea cones were found by Sibilia (*6*) to contain discolored, powdery seeds in some of which the kernel was still sound but in others it was blackened or completely destroyed, its place being taken by a grayish mycelial mat. The fungus was tentatively identified as *Alternaria tenuis*. If this fungus had not penetrated the seed before planting, germination was normal.

Busteed [3] reported the decay of *Pinus nigra* seed by *Physalospora obtusa* in nursery beds. The fungus was found fruiting on the cones and in cone inoculation experiments; ovules were destroyed, and the number of seeds produced was markedly decreased.

Since the experiments of Rathbun-Gravatt had been conducted at room temperatures, the purpose of the present investigation was to study the relation of a somewhat different array of organisms and lower temperature to germination reduction and seed decay. Many of the organisms used were isolated from fresh and old seeds.

ORGANISMS ISOLATED FROM SEEDS

MATERIAL AND METHODS

Organisms were isolated from fresh coniferous seeds, from seeds from nursery beds where germination was a failure and from stratified seeds. These last were stratified in flats in a nursery soil, a greenhouse potting soil, and a pure quartz sand, all maintained, from sowing to completion of germination, alternatingly at 40° and 50° F. during each successive 3-day period. Neither seeds nor substrata were sterilized. Isolations were made at weekly intervals until the stratified seeds started to germinate. The seeds were surface-sterilized with a 1-percent solution of chloramine for 20 minutes, or with $\frac{1}{1000}$, $\frac{1}{500}$, and $\frac{1}{250}$ solutions of bichloride of mercury alone or mixed with 95-percent alcohol, the treatment ranging from 8 to 20 minutes. A saturated solution of bromine water diluted to $\frac{1}{10}$ was also tested, but it proved less satisfactory than the bichloride of mercury and alcohol solution. After sterilization each lot of seed was washed three times with sterile distilled water. The seed was cut open with a flamed instrument. Portions from the inside of the coat, the surface of the endosperm, inside the endosperm, and the embryo were transferred to corn meal, malt, or potato-dextrose agar. Surface-sterilized whole seeds were placed on agar slants as a check on the effectiveness of the sterilization.

RESULTS

Fresh Seeds

Organisms were found in only about 10 percent of the cultures made from fresh seeds of *Pinus banksiana* Lamb., *P. ponderosa* Dougl., and *P. resinosa* Ait., but enough fungi were present to explain spotty decay in nursery beds and the complete loss of whole beds planted with some lots of seed that were examined. *Aspergillus*, bacteria, *Cladosporium*, *Fusarium* spp., *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma*, and several unidentified fungi were isolated. Bacteria and *Fusarium* spp. were isolated most frequently.

[3] Busteed, Robert C. The pathogenicity of physalospora obtusa on pinus nigra. [Unpublished doctor's thesis. Copy on file Ind. Univ. Lib., Bloomington, Ind.]

Seeds From Nursery Beds in Which Germination Had Failed

A majority of the seeds taken from beds in which there was little or no germination were blind or in various stages of decay. As the seeds had been in the soil for some time, a number of organisms were repeatedly isolated from them. It was not possible to state, however, whether these organisms were primary or secondary. A few seeds still appeared sound and of normal color when dissected, but they were usually sterile. Isolations were made from *Picea glauca* (Moench) Voss and from *Pinus caribaea* Morelet, *P. contorta* Dougl., *P. palustris* Mill., *P. ponderosa* Dougl., and *P. taeda* L. The following organisms were isolated from one or more of these species: *Alternaria*, *Aspergillus*, bacteria, *Botrytis*, *Cephalosporium* (?), *Cladosporium*, *Fusarium*, *Helminthosporium* (?), *Mucor*, nematodes, *Penicillium*, *Pestalozzia*, plaster mold, *Rhizoctonia*, *Rhizopus*, *Trichoderma*, *Verticillium*, and several unidentified fungi. *Fusarium*, *Penicillium*, *Rhizopus*, and *Trichoderma* were isolated from *Robinia pseudoacacia* L.; *Penicillium* and *Trichoderma* were found in *Castanea mollissima* Bl. and *C. sativa* Mill.

Stratified Seeds

The results of the isolations from the stratified seeds are presented in table 1. The character of the isolates did not change appreciably from week to week, nor was there a greater number of organisms isolated in any one week. Organisms were isolated from the seeds stratified in the quartz sand fewer times than from those in soil. There was no correlation between the seed quality and the number of isolates.

Table 1.—*Number of times the indicated organisms were isolated from stratified coniferous seeds*

[Seeds surface-sterilized. From sowing to completion of germination, seeds stratified alternatingly at 40° and 50° F. during each successive 3-day period]

Substratum and species	Quality or condition of seed	Organisms isolated (number of times)											
		Aspergillus	Bacteria	*Chartomium globosum* Kze.	*Fusarium moniliforme* Sheld	*Fusarium*	*Mucor*	*Penicillium*	*Rhizopus*	*Thielaria*	*Trichoderma*	Unidentified	Total
Quartz sand:													
Pinus ponderosa Dougl	Good	0	1	1	0	1	0	0	0	0	0	4	7
P. resinosa Ait	do	0	1	0	0	0	0	0	0	0	0	1	2
P. strobus L	do	0	3	0	0	0	0	0	0	0	1	3	7
P. caribaea Morelet	do	0	1	0	1	0	0	0	0	0	0	0	2
Do	Fair	0	2	0	0	0	0	0	0	0	0	1	3
Do	Poor	0	1	0	0	0	0	0	0	0	0	0	1
P. taeda L	Good	0	2	0	0	0	0	0	0	0	0	0	2
Do	Fair	0	1	0	1	0	0	0	0	0	0	1	3
Do	Poor	0	1	0	0	0	0	0	0	0	0	1	2
P. echinata Mill	Good	0	1	0	0	0	0	0	0	0	0	0	1
Do	Fair	0	0	0	0	0	0	0	0	0	0	1	1
Do	Poor	0	1	0	0	0	0	1	0	0	0	0	2
Total		0	15	1	2	1	0	1	0	0	1	12	33

TABLE 1.—*Number of times the indicated organisms were isolated from stratified coniferous seeds*—Continued

Substratum and species	Quality or condition of seed	Organisms isolated (number of times)											
		Aspergillus	Bacteria	*Chaetomium globosum* Kze.	*Fusarium moniliforme* Sheld.	*Fusarium*	*Mucor*	*Penicillium*	*Rhizopus*	*Thielavia*	*Trichoderma*	Unidentified	Total
Greenhouse soil:													
P. ponderosa	Good	0	2	0	0	2	0	0	0	0	0	4	8
P. resinosa	do	0	5	0	0	0	0	0	0	0	0	2	7
P. strobus	do	0	3	0	0	0	0	1	1	0	0	2	7
P. caribaea	do	0	0	0	0	0	0	0	2	0	0	0	2
Do	Fair	0	1	0	0	0	1	0	0	0	0	1	3
Do	Poor	0	1	0	0	0	0	0	1	0	1	0	3
P. taeda	Good	0	2	0	0	0	0	0	0	0	0	2	4
Do	Fair	0	1	0	0	1	0	0	1	0	0	1	4
Do	Poor	0	1	0	0	0	0	0	0	1	0	1	3
P. echinata	Good	0	2	0	0	0	0	0	2	0	1	0	5
Do	Fair	0	2	0	0	0	0	0	1	0	0	2	5
Do	Poor	0	2	0	0	0	0	0	1	0	0	1	4
Total		0	22	0	0	3	1	1	9	1	2	16	55
Nursery soil:													
P. ponderosa	Good	1	2	0	0	2	0	0	0	0	0	5	10
P. resinosa	do	1	0	0	0	0	0	0	1	0	0	0	2
P. strobus	do	0	5	0	0	0	1	0	2	0	0	6	14
P. caribaea	do	0	1	0	0	1	0	1	0	0	0	1	4
Do	Fair	0	1	0	0	0	0	0	1	0	0	1	3
Do	Poor	0	1	0	0	0	0	0	1	0	0	0	2
P. taeda	Good	0	1	0	0	1	0	0	1	0	0	1	4
Do	Fair	0	0	0	0	0	0	0	1	0	1	2	4
Do	Poor	1	0	0	0	1	0	0	0	0	0	0	2
P. echinata	Good	0	2	0	0	0	0	0	1	0	0	1	4
Do	Fair	0	2	0	0	1	0	0	1	0	0	1	5
Do	Poor	0	0	0	0	0	0	0	1	0	1	1	3
Total		3	15	0	0	6	1	1	10	0	2	19	57

INOCULATION EXPERIMENTS

MATERIAL AND METHODS

The cultures of *Alternaria brassicae* var. *microspora* P. Brun., *Pythium* spp., *Rhizoctonia* sp., and *Sphaeropsis ellisii* Sacc. that were used in the inoculation experiments were obtained from the stock cultures of the Division of Forest Pathology. The *Botrytis* culture was obtained from Dr. Charles Brooks, of the Bureau of Plant Industry, and had been isolated from blackberry fruit. The rest of the fungi used (see table 2) were isolated from pine seeds.

For these experiments, 30 cc. of Difco corn-meal agar, one-half concentration of the formula stated on the bottle, plus 15 gm. of agar was poured into Petri dishes. The plates were then inoculated with the fungi to be tested. The growth of the fungi was inhibited to such a degree by the low temperatures used that it was necessary to keep the plates at room temperature for a few days to obtain a good coverage of the agar surface. The plates were then seeded with various pine species and stored at low temperatures as shown in the footnotes of table 2. Experiment 1 was conducted at the lowest temperatures and experiments 4 and 5 at the highest temperatures. When testing organisms producing heavy mycelial mats, two or three small rectangles, approximating 2 inches long and 0.5 inch wide, were cut from the agar of the fungus culture in each plate and the seed

was placed on the glass along the edges of and in contact with the agar, since otherwise the mat would have kept the seed from taking up moisture.

The fungi were reisolated from radicles showing decay.

RESULTS

The average radicle decay in the different types of experiments with each species is given in table 2.

TABLE 2.—*Germination reduction and radicle decay of pines in agar plates inoculated with various fungi at different temperatures*

Experiment, temperature, and inoculating fungus	Germination in uninoculated units and reduction (−) or increase (+) in inoculated units (percentage of total seeds)				Radicle decay (percentage of total radicles)			
	Pinus resinosa [1]	*Pinus ponderosa* [2]	*Pinus banksiana* [3]	Weighted average	*Pinus resinosa*	*Pinus ponderosa*	*Pinus banksiana*	Weighted average
Experiment 1 (32° and 50° F.): [4]								
Botrytis (cinerea?)	0				9			
Sphaeropsis ellisii Sacc.	−10				29			
Uninoculated	87				0			
Experiments 2 and 3 (40°, 50°, and 60° F.): [5]								
Unidentified (No. 114)	−33	−27		−31	8	26		12
Fusarium sp. (No. 132a)	−33	−16		−29	1	9		3
Botrytis (cinerea?)	−27	−27		−27	8	7		8
Plaster mold	−17	−38		−22	2	0		2
Fusarium conglutinans Wr.	−15	−22		−17	31	17		28
Penicillium sp.	−20	−2		−16	0	13		4
Phycomycete (unidentified)	−15	−16		−15	6	13		8
Cylindrocladium sp.	−9	−31		−14	5	0		4
Aspergillus niger Tiegh.	−9	−18		−11	16	16		16
Pestalozzia sp.	−7	−25		−11	4	0		3
Sphaeropsis ellisii	−11	−11		−11	11	27		15
Unidentified (No. 19)	−3	−29		−9	2	0		1
Rhizopus oryzae Went (No. 104) [6]	−7	−18		−9	0	0		0
Rhizoctonia sp.	−3	−27		−8	4	7		4
Alternaria brassicae var. *microspora* P. Brun.	−3	−18		−6	0	0		0
Verticillium	−3	−11		−4	1	3		1
Average of inoculated	−13	−21		−16	6	9		7
Uninoculated	90	87		89	0	0		0
Experiments 4 and 5 (60° and 77° F.): [7]								
Aspergillus niger	−42	−40	−57	−49	0	0	0	0
Pythium ultimum Trow	−27	−13	−48	−33	82	95	100	93
Chaetomium globosum Kze.	−25	−29	−20	−24	0	0	0	0
Pythium debaryanum Hesse	−18	0	−36	−21	78	67	95	82
Unidentified (No. 178)	−7	−2	−33	−14	62	77	100	82
Rhizopus sp. [6 8]	−22	−2	−7	−10	0	0	0	0
Rhizopus oryzae (No. 199) [6]	−18	+2	−11	−9	0	0	0	0
Rhizopus circinans Tiegh. [8]	−18	−2	−7	−8	0	0	0	0
Mucor sp. [8]	−13	−9	−4	−8	0	0	0	0
Thielavia sp.	−16	+4	−9	−7	0	0	0	0
Fusarium sp. (No. 38)	−5	−4	−8	−6	18	32	19	22
Penicillium sp.	−18	+2	−4	−6	0	0	0	0
Unidentified (No. 64)	−9	−2	−8	−7	3	0	3	2
Fusarium sp. (No. 132a)	−7	−11	+3	−4	19	18	36	28
Unidentified (No. 145)	−13	+9	−5	−4	27	19	58	40
Unidentified (No. 179)	−9	+13	−5	−1	0	12	13	9
Unidentified (No. 180)	−9	+18	−2	+1	0	0	0	0
Average of inoculated	−16	−4	−15	−12	18	19	25	22
Uninoculated	89	60	91	82	0	0	0	0

[1] 50 seeds per plate in experiments 1 to 3, and 15 seeds per plate in experiments 4 and 5. Plates in triplicate in each experiment.

[2] 15 seeds per plate. Plates in triplicate in each experiment.

[3] 25 seeds per plate. Plates in triplicate in each experiment.

[4] Seeds sterilized for 20 minutes in 1-percent chloramine solution. From sowing to completion of germination plates stored alternatingly at 32° and 50° F. during each successive 3-day period.

[5] Seeds not sterilized. For 4 weeks after sowing plates stored alternatingly for 1 day at 40° F. and 2 days at 50° and for the next 8 weeks alternatingly for 1 day at 50° and 2 days at 60°.

[6] Tips soft but not discolored.

[7] Seeds sterilized for 12 minutes in 1/250 $HgCl_2$ and washed in 3 changes of sterile water. From sowing to completion of germination plates stored alternatingly for 2 days at 60° F. and 1 day at 77°.

[8] Apparently a different species from *R. oryzae* and *R. circinans*.

Since *Aspergillus, Penicillium,* and *Fusarium* No. 132a were the only three species used in all experiments 2 to 5, no detailed comparisons as to the effects of temperature can be made. However, *Aspergillus, Chaetomium, Mucor, Penicillium, Thielavia,* and three species of *Rhizopus* did not cause radicle decay of any of the species tested at 60° and 77° F.; *Alternaria* and *Rhizopus oryzae* did not cause radicle decay at the lower temperatures of experiments 2 and 3.

Aspergillus decayed radicles of both pines tested, and *Penicillium* decayed radicles of *Pinus ponderosa* but not of *P. resinosa* in experiments 2 and 3.

In experiments 4 and 5 *Pythium debaryanum* and *P. ultimum* caused much radicle decay in all three pines, the only instance known to the writer in which these two species have been directly compared on conifers. *P. debaryanum* was very destructive, but always less so than *P. ultimum* in respect both to radicle decay and germination reduction. In the same experiments the two strains of *Fusarium* used were consistently pathogenic on all species.

In experiments 2 and 3 *Fusarium* No. 132a only slightly affected the radicles of *Pinus resinosa* and caused less radicle decay of *P. ponderosa* than at the higher temperatures, while *Fusarium conglutinans* caused much decay in both species. *Sphaeropsis* caused a moderate amount of loss.

Botrytis, an unidentified phycomycete *Rhizoctonia,* and *Verticillium* caused a small amount of radicle decay in experiments 2 and 3. *Cylindrocladium,* an unidentified species (No. 19, tentatively identified as *Macrosporium*), *Pestalozzia,* and plaster mold caused a slight decay of *Pinus resinosa* radicles but did not affect *P. ponderosa* seedlings in these experiments.

Five of the six unidentified fungi tested caused some radicle decay. No. 64 has been tentatively identified as *Fusarium trichothecioides* Wr. and No. 114 as a mixture of *Fusarium oxysporum* Schlecht. and *Penicillium frequentens* Westling.

As shown in table 2, there was a general decrease in the percentage of germination in practically all inoculated units, even when there was no visible radicle or seed decay. This result was unexpected and requires some further consideration. In experiments 4 and 5, although *Aspergillus niger* did not cause radicle decay, it did cause a marked reduction in germination. So also did *Chaetomium globosum.* Except in one instance in experiments 4 and 5, the fusaria reduced germination, although none of the strains were consistent in respect to the severity of the reduction. The three species of *Rhizopus* and *Thielavia* caused a greater reduction in germination with *Pinus resinosa* than with the other two pines. *Sphaeropsis* did not cause a great reduction with either of the pines on which it was tested.

Temperature exerted a marked influence on the rate of germination in these experiments. In the cultures alternating at 32° and 50° F., initiation of germination was delayed for 10 weeks and the plates finally had to be removed to the laboratory before some of the last seeds would germinate. In experiments 2 and 3 germination was slow and spread over a 3-month period. At the highest temperatures it was completed in approximately 30 days. In general, there was a greater germination reduction in the writer's tests than in the tests by Rathbun-Gravatt (*5*), and the correlation between germination reduction and radicle decay was not so apparent as in her work. The

heavy mycelial mats of some cultures may possibly have reduced germination by reducing the supply of oxygen, and some of the germination reduction may have been caused by a supply of moisture inadequate for both fungus and seed. However, there was evidence in many cases that sufficient moisture persisted throughout the test or that seeds were definitely decayed, indicating that germination reduction was caused by the direct action of the fungus.

There was no significant difference in the susceptibility of the hosts under the conditions of these experiments.

SUMMARY

The frequent recurrence of germination failures in coniferous nursery beds suggests the need for extensive studies to determine the proper means of handling seeds and to learn more of their possible pathogens.

A series of organisms was cultured from the interior of fresh and stratified seeds and from seeds taken from nursery beds in which germination had failed. The seeds were surface-sterilized with a 1-percent chloramine solution and with various concentrations of bichloride of mercury alone and mixed with 95-percent alcohol. Some of these organisms were used in the inoculation experiments reported. Bacteria and fusaria were isolated most frequently. The temperatures employed in the experiments were rather low.

In all species tested the following organisms caused decay of radicles just emerged from the seed coats: *Botrytis* (*cinerea?*), *Fusarium* spp., an unidentified phycomycete, *Pythium debaryanum* Hesse, *P. ultimum* Trow, *Rhizoctonia*, *Sphaeropsis ellisii* Sacc., *Verticillium*, and four unidentified fungi.

The following organisms did not cause a consistent loss of radicles in the various experiments: *Aspergillus niger* Tiegh., *Cylindrocladium*, *Penicillium*, plaster mold, *Pestalozzia*, and two unidentified fungi.

The following fungi failed to attack radicles: *Alternaria brassicae* var. *microspora* P. Brun., *Chaetomium globosum* Kze., *Mucor*, *Rhizopus* sp., *R. circinans* Tiegh., *R. oryzae* Went, *Thielavia*, and one unidentified fungus.

There was a general decrease in the percentage of germination in practically all inoculated units, even when there was no visible radicle or seed decay. This reduction in germination was greater than in earlier published tests conducted at room temperatures.

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THE PERSONAL ELEMENT AND LIGHT AS FACTORS IN THE STUDY OF THE GENUS FUSARIUM[1]

By L. L. HARTER

Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Results previously published[2] suggested that some of the difficulty experienced by different investigators in identifying species of *Fusarium* was due, in part at least, to a lack of uniformly controlled environmental conditions. Among other things it was shown (1) that cultures grown in daylight or in artificial light produced significantly longer conidia than those grown in the dark; (2) that the ratio of macroconidia to microconidia was greatest in the light and least in the dark; (3) that sporodochia and pionnotes were more copious in the light than in the dark; (4) that vegetative growth was most abundant in the dark; (5) that exposure of cultures to light for the first few days after inoculation was more influential in producing macroconidia and conidia of greatest length than similar exposure during the later period of growth; and (6) that statistically significant differences in distribution of conidia of various lengths were recorded when different investigators measured conidia from the same microscopic preparation.

The results referred to above suggested the desirability of further investigations of the inconsistencies and variations that may be expected from the use of the present cultural methods. In addition to those conditions already mentioned that influence the behavior of different species of *Fusarium* in culture, it is important to acquire some knowledge of (1) the homogeneity among different tubes of cultures of the same organisms grown under supposedly identical conditions on the same medium, and the variation among different microscopic mounts from the same spore suspension; (2) the variation in distribution of different lengths of three-, four-, and five-septate conidia of different species grown in light and in darkness on different culture media; (3) the influence of light and darkness on the septations of the conidia; (4) the influence of the substrata on septation of the conidia; (5) the number of spores that should be measured in order to obtain a dependable value of mean length; and (6) the personal element of error due to different investigators.

MATERIAL AND METHODS

SPECIES OF FUSARIUM

Two species of *Fusarium* (*F. martii* var. *pisi* F. R. Jones and *F. bulbigenum* var. *batatas* Wr.) have been employed in this study. Inasmuch as these investigations were practically an extension of

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[2] HARTER, L. L. INFLUENCE OF LIGHT ON THE LENGTH OF THE CONIDIA IN CERTAIN SPECIES OF FUSARIUM. Amer. Jour. Bot. 26: 234-243. 1939.

previous work, use of the same species was believed to be desirable. Both were known to produce pionnotes and sporodochia freely in certain media and to respond readily to different degrees of illumination.

Fusarium martii var. *pisi*, a representative of the section Martiella, is the cause of root rot of pea (*Pisum sativum* L.) and produces green pionnotes or sporodochia on cooked potatoes, 2-percent potato-dextrose agar, cooked green bean pods, corn-meal agar, and certain other media. By frequent transfers to suitable media, it has been maintained in "high culture" for several years—a condition highly essential for investigations of this type.

Fusarium bulbigenum var. *batatas*, a representative of the section Elegans, is a vascular parasite and the cause of the wilt or stem rot of sweetpotatoes (*Ipomoea batatas* (L.) Lam.). It produces copious pionnotes and sporodochia on corn-meal agar, 2-percent potato-dextrose agar, and other substrata when first isolated. This species was more difficult to maintain in high culture than *Fusarium martii* var. *pisi*.

EXPERIMENTAL PROCEDURE

The methods of experimentation were substantially the same as those published in a previous article.[3] Only the methods that differed from those previously described and that were introduced to meet the requirements of specific experiments will be discussed. Solar irradiation was generally employed, although artificial light was used in a few cases.

Cultures grown in the light and in the dark were identical in every respect, except that those culture tubes from which the light was excluded were wrapped in several thicknesses of black paper; the cotton plug was not wrapped. Following inoculation of the media, the tubes were placed on the ledge of a north window, where the temperature varied from 15° to 24° C., or in an artificially lighted incubator. Under the conditions of the experiments, sporodochia began to develop in about 5 to 8 days, and in 10 days pionnotes were frequently present. The cultures appeared to be mature in about 14 days, but the spore counts or measurements were not made until the cultures were 18 days old.

RESULTS

DIFFERENCES IN RESULTS DUE TO INDIVIDUAL WORKERS

Two investigators familiar with pathological technique and the technique of measuring conidia participated in this experiment. Cultures of *Fusarium martii* var. *pisi* on 2-percent potato-dextrose agar and *F. bulbigenum* var. *batatas* on corn-meal agar were grown in the light for 18 days on the ledge of a north window. At the end of that time, a thin sowing of conidia was made on a microscopic slide and the edges of the cover glass were sealed with paraffin to prevent evaporation of the mounting fluid and movement of the spores. Each participant measured 500 three-septate conidia to the nearest line on the eyepiece micrometer. The participants were instructed to begin at one side of the microscopic preparation and to move the slide in one direction in order not to cover the same field more than once. Each

[3] See footnote 2.

spore measurement was later converted into microns. The spaces on the eyepiece micrometer are equivalent to 2.5μ. In the tables, there is an interval of 2.5μ between classes and the number of conidia in each class is recorded.

The 500 measurements of each participant were divided into 10 groups, the first 50 measurements constituting the first group, the second 50 the second group, the third 50 the third group, and so on, which made it possible to calculate statistically the number, in multiples of 50 conidia, that should be measured to obtain mean values that would show no significant difference from means based on somewhat larger numbers.

The frequency distribution of the 10 groups, based on spore length in microns, is shown in tables 1 and 2. The mean length, standard error of the mean, and the coefficient of variation have been calculated from these measurements. Table 1 shows that B recorded more conidia in the 27.5μ class than A (11:3). On the other hand, A recorded more conidia in the 50.0μ class than B (12:5). In the case of both A and B, a large percentage of the conidia fell into classes 37.5μ to 45.0μ. If the mean of the total number of spore measurements of each participant is compared, a small but inconsequential significant difference is found.

The following conclusions may be drawn from A's data: (1) A comparison with the mean of the 500 spore measurements of the mean of each of the 10 groups of 50 spore measurements shows that none are significantly different; (2) when the mean of the first group of 50 measurements is compared with the mean of each of the other 50-spore populations, it is found that it is not significantly different; (3) if the means of 50, 100, 150, 200, etc., spore measurements are compared with the mean of 500, no significant difference is found.

The following conclusions may be drawn from B's data: (1) A comparison of the mean of 500 spore measurements with the mean of each of the component groups of 50 spore measurements shows that 1 is significantly different; (2) when the means of the other 50-spore populations are compared with the mean of the first 50 spore measurements, 2 are found significantly different; (3) if the means of 50, 100, 150, 200, etc., spore measurements are compared with the mean of 500, 1 (100) is found significantly different. The 100 population was obtained by adding the first and second columns, the 150 by adding the first 3 columns, and the 200 by adding the first 4, etc. It should not be presupposed, however, that the results from a single tube would necessarily be identical with, or even representative of, other cultures of the same organism.

Table 2 reveals about the same facts as table 1. There are some minor significant differences in most cases between the data obtained by A and B. There is a significant difference between the two observers from the first 50 spore measurements. The results of A and B taken separately show a high degree of consistency. No significant difference was found between 50, 100, 150, and 200 spore measurements of either A or B, which indicates that under the conditions of these experiments 50 spore measurements would be about as good as 100, 150, or 200, although the standard error naturally becomes slightly less with the higher number of conidia measured.

TABLE 1.—*Frequency distribution of length measurements made by observers A and B of 10 samples of 50 spores each of 3-septate conidia of Fusarium martii var. pisi grown on 2-percent potato-dextrose agar*

Length measurement (μ)	Distribution of length measurements as determined by A or B for sample No. —																					
	1		2		3		4		5		6		7		8		9		10		Total	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
27.5 ..... number						1	2	1		2		2	1	2		1		1		1	3	11
30.0 ..... do		2	1			4	1	1	1	4		1	1	1		2	2	1		1	6	17
32.5 ..... do	2	1	2	2	3	2	1	2	4	3	2	3	1	5	5	4	2	1	3	5	25	28
35.0 ..... do	4	3	5	2	5	5	4	6	5	5	2	5	2	5	4	4	5	4	4	3	40	42
37.5 ..... do	6	9	4	5	4	8	6	12	6	9	10	3	5	9	8	5	10	10	8	8	67	78
40.0 ..... do	5	5	15	10	14	6	15	10	11	11	16	13	11	13	10	10	11	13	11	9	119	100
42.5 ..... do	16	17	8	13	11	9	12	9	11	9	10	6	13	10	9	17	8	14	17	15	115	119
45.0 ..... do	12	9	13	12	8	9	7	6	7	5	7	11	11	3	12	6	8	5	3	7	88	73
47.5 ..... do	4	2	1	5	4	6		3	5	1	2	5	4	2		1	3	1	2	1	25	27
50.0 ..... do	1	2	1	1	1		2			1	1	1	1		2		1		2		12	5
Total ..... do	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	500	500
Mean length ..... μ	41.8	41.1	40.8	42.0	41.0	40.0	40.1	39.5	40.4	38.6	40.7	40.5	41.5	38.7	40.5	39.7	40.2	39.8	40.6	39.7	40.8	40.0
S. E.[1] ..... μ	±0.57	±0.62	±0.59	±0.55	±0.59	±0.76	±0.65	±0.62	±0.62	±0.72	±0.50	±0.75	±0.62	±0.65	±0.63	±0.64	±0.61	±0.55	±0.56	±0.63	±0.19	±0.21
C. V.[2] ..... percent	9.6	10.5	10.1	9.2	10.1	13.3	11.3	11.0	10.7	13.1	8.6	13.0	10.5	11.8	10.9	11.3	10.6	9.7	9.7	11.0	11.0	12.0

Length measurement (μ)	1—2		1—3		1—4		1—5		1—6		1—7		1—8		1—9	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Cumulative data:																
Total ..... number	100	100	150	150	200	200	250	250	300	300	350	350	400	400	450	450
Mean length ..... μ	41.3	41.6	41.2	40.1	40.9	40.7	40.8	40.3	40.8	40.3	40.9	40.1	40.9	40.0	40.8	40.0
S. E.[1] ..... μ	±0.41	±0.42	±0.34	±0.26	±0.30	±0.33	±0.27	±0.31	±0.24	±0.28	±0.23	±0.26	±0.21	±0.24	±0.20	±0.22
C. V.[2] ..... percent	9.9	10.1	10.1	7.9	10.4	11.5	10.5	12.2	10.2	12.0	10.5	12.0	13.6	12.0	10.4	11.7

[1] Standard error of mean.

[2] Coefficient of variation.

TABLE 2.—*Frequency distribution of length measurements made by observers A and B of 10 samples of 50 spores each of 4-septate conidia of*

TABLE 2.—*Frequency distribution of length measurements made by observers A and B of 10 samples of 50 spores each of 4-septate conidia of Fusarium bulbigenum var. batatas grown on corn-meal agar*

Length measurement (μ)	Distribution of length measurements as determined by observers A and B for sample No.—																					
	1		2		3		4		5		6		7		8		9		10		Total	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
30.0 number																						
32.5 do															1						1	
35.0 do			1	2	3				3		1	2	2	2							10	6
37.5 do	12	1	8	3	7	3	7	5	6	6	6	4	6	5	4	5	7	1	7	3	70	36
40.0 do	10	8	16	11	11	17	19	7	13	6	15	11	18	12	10	12	14	13	8	5	134	102
42.5 do	19	18	12	22	17	17	11	19	17	21	15	19	10	18	20	19	16	19	18	15	155	187
45.0 do	6	16	10	11	8	10	6	11	7	10	9	9	7	11	9	9	9	9	8	17	79	113
47.5 do	2	4	2	1	3	3	6	5	4	7	3	3	4	1	5	4	3	6	7	5	39	39
50.0 do	1	3	1				1	3			1	2	2	1	1	1	1		2	4	10	14
52.5 do					1								1					1			2	1
55.0 do																		1		1		2
Total do	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	500	500
Mean length μ	41.4	43.6	41.6	42.0	41.7	42.1	41.9	43.1	41.5	42.8	41.9	42.0	41.9	41.9	42.5	42.4	42.0	43.3	42.8	41.6	41.9	42.5
S.E μ	±0.32	±0.39	±0.44	±0.37	±0.5	±0.35	±0.46	±0.45	±0.45	±0.40	±0.44	±0.52	±0.54	±0.42	±0.45	±0.41	±0.42	±0.45	±0.48	±0.48	±0.14	±0.14
C. V percent	5.4	6.2	7.4	6.2	8.4	5.8	7.7	7.3	7.6	6.5	7.3	8.7	9.0	7.0	7.4	6.8	7.0	7.3	7.8	8.1	7.6	7.3

	1—2		1—3		1—4		1—5		1—6		1—7		1—8		1—9	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Cumulative data:																
Total number	100	100	150	150	200	200	250	250	300	300	350	350	400	400	450	450
Mean length μ	41.5	42.8	41.6	42.6	41.7	42.7	41.6	42.7	41.6	42.6	41.7	42.5	41.8	42.5	41.8	42.6
S. E μ	±0.30	±0.27	±0.27	±0.22	±0.23	±0.20	±0.20	±0.18	±0.21	±0.18	±0.18	±0.17	±0.17	±0.15	±0.18	±0.15
C. V percent	7.2	6.3	7.8	6.3	7.7	6.6	7.7	6.7	8.6	7.2	8.2	7.5	8.1	7.1	9.0	7.5

The mean of 500 conidia measured by B (table 2) and the mean of the same number measured by A differ by an amount that is significant but not important. An examination of the 10 paired means of A and B (table 2) shows that the means of B are higher than those of A, but in table 1 this difference is reversed. One possible explanation for this difference might be suggested. Both observers were instructed to introduce no fractional readings on the eyepiece micrometer. If the length of the conidium extended beyond the midway point of the scale, it was to be recorded as extending to the number above; if it was less than a half, it was to be dropped. When the end of the conidium rested on the halfway point the observer was obliged to use his own judgment, and it is not unlikely that one observer consistently dropped the half fractions and the other read to the next number above.

HOMOGENEITY OF CONIDIAL SEPTATION

The ratio or percentage of the number of conidial septations on different culture media has been employed by some investigators in the description of species of *Fusarium*. The value of such ratios as taxonomic characters would seem to depend largely on the constancy with which any given ratio of septations can be expected to occur in spores from cultures grown under the same environment and on the same medium. If a constant ratio could be expected in all cultures thus grown on some standard medium, the variations obtained when the organism was grown on different media would be less important.

To determine the homogeneity of conidial septation in samples taken from different culture tubes, all of the tubes were inoculated from the same spore suspension in water and exposed to the light of a north window. Great care was taken to eliminate as nearly as possible those variations that might result from culturing and mechanical handling. At the end of 18 days of growth the spore masses (pionnotes) in each culture tube were thoroughly mixed. A portion of this mass was then transferred to a few cubic centimeters of water and stirred. From this suspension a thin sowing of conidia was made on a microscopic slide, and the septation of all the spores in a single field of the microscope was counted; the position of the slide was then changed to bring another sample population of conidia into view. This was repeated as often as necessary to accumulate counts on as large a number of individuals as might be required. The number of conidia in a single field of the microscope was intentionally made small so that there would be no confusion in classifying each spore according to the number of septations. Usually the counts were continued until 100 or 200 three-septate conidia were found, the number of two-, four-, and five-septate spores being registered accordingly.

HOMOGENEITY OF SEPTATION IN DIFFERENT MOUNTS FROM THE SAME CULTURE

The ratio of three-, four-, and five-septate conidia in 100 spores taken at random was determined for different mounts from one culture tube of *Fusarium martii* var. *pisi* and *F. bulbigenum* var. *batatas*. The results are shown in table 3. A similar method of procedure was employed when different tubes were compared.

The χ^2 values in table 3 indicate that there is no significant deviation from the mean for the 10 mounts in the ratio of three-, four-, and

five-septate conidia for any one of the 10 microscopic preparations of cultures of *Fusarium martii* var. *pisi* grown on either 2-percent potato-dextrose agar or cooked green bean pods. A similar conclusion can be drawn from the data of table 4, which were obtained from the use of a different organism, *F. bulbigenum* var. *batatas*. These data show that a satisfactory homogeneous preparation can be prepared by suspending the conidia in water after thoroughly mixing them in the culture tube.

TABLE 3.—*Ratio of 3-, 4-, and 5-septate conidia*[1] *in 10 microscopic mounts from a spore suspension of a single culture of Fusarium martii var. pisi, grown on two different culture media*

Medium	Septation	Microscopic mount No.— 1	2	3	4	5	6	7	8	9	10	Total
2-percent potato-dextrose agar	3	70	75	74	72	71	69	74	74	70	78	727
	4	27	24	25	27	28	30	25	24	30	22	262
	5	3	1	1	1	1	1	1	2	0	0	11
x^2		3.40	0.35	0.17	0.12	0.25	0.83	0.16	0.94	0.65	1.06	7.94
Cooked green bean pods	3	69	72	75	64	78	74	70	72	71	72	717
	4	28	26	23	34	20	24	29	27	27	27	265
	5	3	2	2	2	2	2	1	1	2	1	18
x^2		0.26	0.13	0.63	2.97	2.16	0.33	1.0	0.46	0.18	0.46	8.58

[1] 100 conidia observed in each mount.

TABLE 4.—*Ratio of 3-, 4-, and 5-septate conidia*[1] *in 10 microscopic mounts of a spore suspension from each of 2 culture tubes of Fusarium bulbigenum var. batatas grown on corn-meal agar*

Tube No.	Septation	Microscopic mount No.— 1	2	3	4	5	6	7	8	9	10	Total
1	3	23	24	20	23	26	19	19	20	23	21	218
	4	20	19	22	19	16	23	24	22	21	21	207
	5	7	7	8	8	8	8	7	8	6	8	75
x^2		0.34	0.53	0.26	0.24	1.91	0.65	0.92	0.26	0.41	0.36	5.88
2	3	11	14	12	13	7	13	13	18	14	8	123
	4	20	17	22	17	18	20	20	17	17	17	185
	5	19	19	16	20	25	17	17	15	19	25	192
x^2		0.28	0.37	1.26	0.49	4.17	0.41	0.41	3.68	0.37	3.37	14.81

[1] 100 conidia observed in each mount.

A study of the tables discloses several interesting facts. Table 3 shows a remarkable uniformity in the ratio of the total three-, four-, and five-septate conidia grown on 2-percent potato-dextrose agar and on cooked green bean pods. On the other hand, table 4 shows a somewhat greater difference between tubes that were handled by the same method. Five hundred spores from each tube were classified according to septation, and it was found that the three-septate conidia predominated in tube 1 and the five-septate in tube 2; likewise, the five-septate conidia were fewest in tube 1, the three-septate the fewest in tube 2. The results indicate that, although there seems to be no significant difference between preparations from the same culture, the

various culture tubes may differ very greatly. The differences or the similarities between tube cultures will be more clearly shown in some of the following tables.

Table 5 shows a high degree of homogeneity in the ratio of three-, four-, and five-septate conidia in the two series of cultures. In no case is there any significant deviation from the mean.

TABLE 5.—*Ratio of 3-, 4-, and 5-septate conidia in different culture tubes of Fusarium bulbigenum var. batatas grown on corn-meal agar*

Series No.[1]	Septation	Tube No. 1	2	3	4	5	6	7	Total
1	3	57	52	48	72	39	61		329
	4	71	89	100	100	98	100		558
	5	100	100	94	99	100	81		574
	Total	228	241	242	271	237	242		1,461
	χ^2	4.80	0.49	1.41	2.62	5.01	3.48		17.81
2	3	39	39	34	39	32	31	36	250
	4	47	37	38	41	37	45	42	287
	5	14	24	28	20	31	24	22	163
	Total	100	100	100	100	100	100	100	700
	χ^2	4.82	0.71	1.25	0.60	3.33	1.03	0.32	12.07

[1] Series 2 was conducted about 3 months later than series 1; otherwise they were alike.

The ratio of septations of the conidia in some series was very homogeneous; in others it deviated greatly from the mean (tables 6 and 7). Three culture tubes (table 6, series 1) showed χ^2 values of 12.46, 9.37, and 14.20, respectively, which, with 2 degrees of freedom, is much beyond the 5-percent point. The other 7 tubes in the series were not significantly different from the mean for the 10 tubes. In table 6, the χ^2 value exceeds the 5-percent point in all cultures in series 2, and in one culture in series 3. Table 7 also shows a lack of homogeneity in the ratio of conidial septation in both series 1 and 2.

TABLE 6.—*Ratio of 3-, 4-, and 5-septate conidia in different culture tubes of Fusarium martii var. pisi grown on 2-percent potato-dextrose agar*

Septation	Tube No. (series 1)[1]— 1	2	3	4	5	6	7	8	9	10	Total
3	88	69	87	85	78	85	91	76	94	68	821
4	12	30	13	14	22	15	9	23	6	32	176
5	0	1	0	1	0	0	0	1	0	0	3
Total	100	100	100	100	100	100	100	100	100	100	1,000
χ^2	2.21	12.46	1.49	0.98	1.31	0.48	4.90	2.27	9.37	14.20	47.66

Septation	Tube No. (series 2)[1]— 1	2	3	4	5	6	Total	Tube No. (series 3)[1]— 1	2	3	4	5	6	Total
3	100	100	100	100	100	100	600	100	100	100	100	100	100	600
4	112	23	22	20	31	81	289	33	49	22	30	33	66	233
5	13	2	4	4	2	5	30	2	3	1	1	1	2	10
Total	225	125	126	124	133	186	919	135	152	123	131	134	168	843
χ^2	21.73	12.07	11.66	13.74	6.06	12.62	76.88	1.60	2.14	6.13	1.75	0.66	12.32	24.60

[1] Series 1, 2, and 3 are identical except that they represent cultures grown at different dates.

TABLE 7.—*Ratio of 3-, 4-, and 5-septate conidia in different culture tubes of Fusarium martii var. pisi grown on cooked green bean pods*

Septation	Tube No. (series 1)—										Total
	1	2	3	4	5	6	7	8	9	10	
3	72	53	68	71	79	80	62	90	72	76	723
4	26	41	27	26	20	20	34	10	22	21	247
5	2	6	5	3	1	0	4	0	6	3	30
Total	100	100	100	100	100	100	100	100	100	100	1,000
χ^2 values.	0.40	14.28	2.14	0.03	2.84	1.71	5.3	13.8	3.30	0.74	44.54

Septation	Tube No. (series 2) [1]—						Total
	1	2	3	4	5	6	
3	100	100	100	95	100	100	595
4	90	33	53	100	54	72	402
5	21	5	12	31	11	29	109
Total	211	138	165	226	165	201	1,106
χ^2 values	3.92	20.25	3.35	13.03	3.71	5.39	49.65

[1] Series 2 is the same as series 1, except that the cultures were grown at a different date.

The data indicate that cultures of *Fusarium bulbigenum* var. *batatas* are more uniform (table 5) than cultures of *F. martii* var. *pisi* (tables 6 and 7). *Fusarium bulbigenum* var. *pisi* on corn-meal agar gave no cultures that were significantly different from the mean. Whether or not this would hold true for a large number of cultures and on other media could be determined only by additional trials and experimentation.

Three conclusions may be drawn from these data: (1) That different cultures presumably alike may vary greatly in the ratio of three-, four-, and five-septate conidia; (2) that while three-septate conidia may predominate in one culture, four- or five-septate may predominate in others; and (3) that from a taxonomic standpoint it is unsafe to draw any general and sweeping conclusions based on a few cultures.

INFLUENCE OF IRRADIATION ON SEPTATION OF CONIDIA

In a previous publication [4] it was shown that irradiation of the cultures increased the length of the conidia as compared with that of conidia produced in the dark. Its effect on the number of septations was noted at that time, but extensive data were not taken. The methods employed in these later studies were essentially the same as those described in the present paper, and the same two species of *Fusarium* were used, viz, *F. martii* var. *pisi* and *F. bulbigenum* var. *batatas*. *F. martii* var. *pisi* was cultured on 2-percent potato-dextrose agar and cooked green bean pods and grown in a culture chamber at 25° C. The cultures grown in the light were irradiated with a 200-watt Mazda lamp at a distance of 26 inches. When light was to be excluded the cultures were wrapped in black paper, only the cotton plug being left exposed, but in every other respect cultures grown in the light and in the dark were identical. *F. bulbigenum* var. *batatas* was grown on corn-meal agar, and one set of cultures was

[4] See footnote 2.

exposed to solar irradiation on the ledge of a north window. The dark cultures were wrapped in several thicknesses of black paper, except that the cotton plug was left free for the exchange of air.

Both organisms responded readily to solar irradiation. Pionnotes and sporodochia were produced more abundantly in the light than in the dark. When the organisms were grown in the dark, however, there was a larger production of mycelium.

Data were obtained from *Fusarium martii* var. *pisi* by combining the results from five and from six cultures grown on 2-percent potato-dextrose agar and cooked green bean pods, respectively, and from *Fusarium bulbigenum* var. *pisi* by combining the results from six cultures grown on corn-meal agar. Spore mounts were made as previously described, and the number of septations was determined for 1,000 and 1,200 spores produced by cultures on the various media grown in darkness and in light. The results are shown in table 8.

Table 8 shows that irradiation of the cultures of both species increased the number of septations. In all cases there was a much larger number of one- and two-septate conidia in the dark than in the light cultures. The highest percentage of four- and five-septate conidia occurred in cultures exposed to light, irrespective of the medium used.

TABLE 8.—*Effect of light and darkness on conidial septation*

Septation	Distribution of 1- to 5-septate conidia of—					
	Fusarium martii var. *pisi*				*Fusarium bulbigenum* var. *batatas*	
	Grown on cooked green bean pods under—		Grown on 2-percent potato-dextrose agar under—		Grown on corn-meal agar under—	
	Light	Darkness	Light	Darkness	Light	Darkness
1	26	221	14	114	0	925
2	24	138	3	142	0	64
3	1,035	839	895	744	407	187
4	115	2	88	0	576	24
5	0	0	0	0	217	0
Total	1,200	1,200	1,000	1,000	1,200	1,200

DISCUSSION

Differences in results and conclusions due to individual workers cannot be eliminated entirely. With respect to the taxonomy of fungi and in particular of the genus *Fusarium* the personal factor is significantly important. Some of the results obtained by two investigators measuring conidia from the same microscopic mount were found to be significantly different, though not highly so, when treated statistically. If a significant difference, though small, occurs under conditions as uniform as those discussed above, it is not surprising that irreconcilable differences occur when the work is performed with little or no attention to uniformity of method.

A homogeneous dispersion of conidia of the two species studied was made from a single culture of the different media employed in which the proportion of three-, four-, and five-septate conidia in 10 different

mounts did not vary enough from the mean for the differences to be significant (tables 3 and 4).

It is evident that no general conclusions can be drawn for all species of *Fusarium* from the results obtained for one or two species alone. *Fusarium bulbigenum* var. *batatas* (table 5) was fairly consistent in its behavior when grown under uniform conditions, since there was no considerable deviation from the mean and none of the χ^2 values exceeded the 5-percent point. *F. martii* var. *pisi*, however, yielded large differences between cultures that appeared to be very uniform in growth, which suggests that enough cultures must be studied for the variations between tubes in respect to septations and spore sizes to be smoothed out.

The results indicate that light was an important factor in the production of what is known as "high culture" and in increasing the size and number of septations of the conidia. A small number of septations (1 and 2) predominated in cultures grown in the dark, and a larger number (3, 4, and 5) predominated in those grown in the light. No five-septate and very few four-septate conidia were found in any of the cultures grown in the dark, whereas in cultures grown in the light they were plentiful, especially in the case of *Fusarium bulbigenum* var. *batatas*, which consistently produces conidia having several septations.

SUMMARY

That different investigators measuring conidia of the same species of *Fusarium* from the same microscopic preparation may secure significantly different results is shown when these results are treated statistically. Groups of 50 spore measurements from the same culture may vary considerably, but 150 to 200 measurements constitute a fairly safe sample of a population if the spore suspensions are carefully prepared and mounted.

There was no significant difference in the ratio of three, four, and five septations of the conidia in different microscope mounts made from the same spore suspension. Highly significant differences, however, sometimes occurred between culture tubes of the same organism when grown on the same medium. A single tube culture cannot be assumed to be representative of a number of tube cultures.

Light has an important influence on the production of conidial septation in certain species of *Fusarium*. A larger number of septations predominated in cultures exposed to the light than in those grown in the dark; on the other hand, a larger number of one- and two-septate conidia predominated in cultures grown in the dark than in those grown in the light. Four- and five-septate conidia were few or entirely lacking in cultures grown in the dark, but they were plentiful in irradiated cultures.

PREDICTION OF CULL FOLLOWING FIRE IN APPALACHIAN OAKS[1]

By GEORGE H. HEPTING[2]

Associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The two most important causes of butt defect in eastern hardwoods are rot that develops from fire wounds and rot that starts in the parent stumps of sprouts and spreads into the new sprouts (*5*).[3] Butt rot has long been recognized as one of the major harmful effects of fire in hardwoods, but only recently have attempts been made to discover means of estimating and predicting the extent of such losses other than by ocular estimate. Improved means of appraising fire damage should lead to better allocation of fire-protection funds and to more equitable settlement of damage claims. A valid method for predicting cull following fire should also help guide salvage policy and marking practice in both timber sales and stand improvement work.

Earlier investigations of decay following fire in Delta hardwoods (*2*) and Appalachian hardwoods (*3*) suggested possible correlation between butt cull and a number of measurable factors, including age of tree, number of years since the fire, diameter of the tree when wounded, and width of wound. The present study was designed to incorporate those factors most highly correlated with butt cull and most easily measurable in the field into a statistical mechanism by which the amount of cull that will develop in oaks wounded by fire in the Appalachian mountain region can be predicted. This work is one phase of an extensive investigation of fire effects that is being carried on at the Appalachian Forest Experiment Station. The characteristics of the forests in the region where this work is centered, with particular regard to the causes and amount of cull due to heart rot, have been described by Hepting and Hedgcock (*3*).

METHODS

The field data were collected during 1937 and 1938 on 11 commercial logging operations distributed as shown in figure 1. The number of trees examined on any one operation depended upon the size and speed of the logging job, the number of species on the area, and range in wound sizes and ages available. The crew followed as closely as possible behind the cutters, examining each stump for basal wounds, both healed and open. Where there was evidence of basal wounding,

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[2] Special acknowledgment is due Prof. F. X. Schumacher, of Duke University, for direction in the analysis of the data, and C. A. Abell, of the California Forest Experiment Station, formerly of the Appalachian station, and G. M. Jemison, of the Appalachian station, for their advice and cooperation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 119.

a record was made of original width of wound at stump height, stump diameter at time of wounding, wound age (years since the fire), tree age, diameter at breast height, and volume of cull that resulted from the wound in board and cubic feet. Where the decay following wounding resulted in the complete loss of a section 6 feet or more in length, the cull section was scaled as a separate log. The volume of cull sections less than 6 feet in length was computed as a proportion of the volume of a 16-foot butt log. Logs were scaled by the International Log Rule. Rot not extensive enough to result in long butts was scaled out according to standard United States Forest Service instructions (*7*).

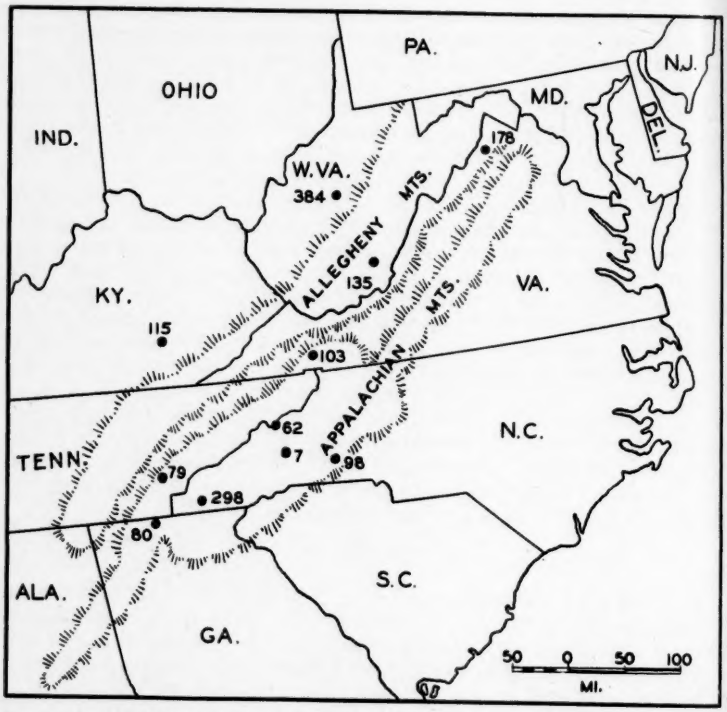

FIGURE 1.—Location of study areas and the number of trees analyzed in each area.

Occasionally rot was evident in a stump that had no indication of basal wounding. Such decay could usually be traced to a low healed wound that did not reach stump height, although in some cases the rot developed in the roots and worked up, and in some sprout trees the decay undoubtedly originated in the parent stumps. There were also cases in which severe decay or hollowing made it impossible to determine the width or age of the wound that led to the decay and sometimes obliterated the wound entirely. Trees upon which only fragmentary data or data requiring an undue amount of estimation

could be obtained, such as some of the types described above, were not recorded. The proportion of trees rejected was small, and the resulting bias was slight.

Although the original height of a basal wound affects the amount of cull that will develop, this factor could not be measured on most trees because it would have been necessary to split the butt logs and that could have been done only with cull sections. Wound width was therefore the only criterion of wound size obtained.

During 1937 the field work was confined to white oak on two stave operations. Since such trees were cut into short lengths, the amount of decay could be accurately measured. This work resulted in the standardization of methods, and in 1938 the work was extended to the other oaks, yellow poplar, and basswood on saw-timber operations. To date, sufficient data have been accumulated for the oaks only, and this paper will be confined to that group. The species studied were white oak (*Quercus alba* L.), chestnut oak (*Q. montana* Willd.), red oak (*Q. borealis maxima* (Marsh.) Ashe), black oak (*Q. velutina* LaM.). and scarlet oak (*Q. coccinea* Muench.).

DEVELOPMENT OF THE EQUATION

Preliminary analyses had established that the two measurable factors most highly correlated with butt-cull volume were wound width and wound age. In order to explore the interrelationships among these three variables, the data were grouped into broad classes of wound width and age and plotted as shown in figure 2. This

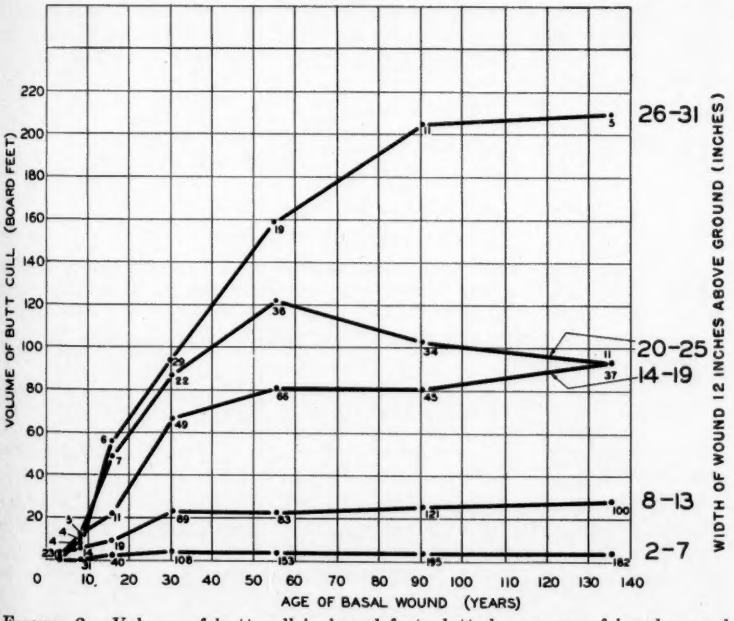

FIGURE 2.—Volume of butt cull in board feet plotted over age of basal wound for different wound widths. This figure is based on the raw data, combining all species of oak. The numbers beside the points indicate the number of trees on which each point is based.

figure is based upon the data from 892 white oaks, 297 chestnut oaks, 157 red oaks, 146 black oaks, and 47 scarlet oaks. It clearly indicates that under average conditions butt rot does not continue to extend indefinitely, but ceases after a certain number of years, depending in part upon the wound width. To provide a means of estimating cull for different wound widths and numbers of years following burning, it seemed desirable to express the relation of butt cull to wound width and wound age in the form of an equation. Since figure 2 indicates little increase in cull beyond 60 years except in the largest wounds, the equation was fitted only to the data through 60 years. It was thus possible to express the relation of cull to wound age in the form of a parabola. A curve expressing the relation of cull to wound width for the data up to 60 years indicated that cull increased with wound width at an increasing rate for narrow wounds, the rate becoming more or less constant for wounds of medium width and decreasing for wide wounds.

It thus appeared that the relation of cull to wound age could be expressed by an equation containing the first and second powers of age as follows:

$$Y=a+b_1A+b_2A^2 \tag{1}$$

where

Y=cull in board feet
a=a constant
b_1, b_2=regression coefficients
A=wound age

Analysis of very young wounds showed that at least 3 years must elapse before there is measurable cull due to rot behind fire wounds. Therefore, the condition is imposed upon equation (1) that when $A=3$, $Y=0$, as follows:

$$\begin{aligned} 0&=a+3b_1+9b_2 \\ a&=-3b_1-9b_2 \end{aligned} \tag{2}$$

Now substituting the value for a in equation (2) for a in equation (1):

$$\begin{aligned} Y&=-3b_1-9b_2+b_1A+b_2A^2 \\ Y&=b_1(A-3)+b_2(A^2-9) \end{aligned} \tag{3}$$

As already mentioned, the relation of Y to A depends upon wound width (W), and because of the shape of the wound width-cull curve it will take three powers of W to express this relation. Thus b_1 and b_2, the terms denoting the slope of the wound age-cull curve, may be expressed as follows:

$$\left.\begin{aligned} b_1&=m+c_1W+c_2W^2+c_3W^3 \\ b_2&=n+d_1W+d_2W^2+d_3W^3 \end{aligned}\right\} \tag{4}$$

where m and n are constants, and c_1, c_2, c_3, d_1, d_2, and d_3 are regression coefficients.

Analysis of more than 1,000 wounds less than 4 inches in width showed that practically no cull resulted from wounds 3 inches or less in width measured about 12 inches above ground. Thus the condition is imposed upon equations (4) that when $W=3$, b_1 and $b_2=0$:

$$\left.\begin{aligned} 0&=m+3c_1+9c_2+27c_3 \\ m&=-3c_1-9c_2-27c_3 \\ \text{similarly } n&=-3d_1-9d_2-27d_3 \end{aligned}\right\} \tag{5}$$

Now substituting equations (5) for m and n, respectively, in equations (4):

$$\left.\begin{aligned} b_1 &= -3c_1 - 9c_2 - 27c_3 + c_1W + c_2W^2 + c_3W^3 \\ b_1 &= c_1(W-3) + c_2(W^2-9) + c_3(W^3-27) \end{aligned}\right\} \quad (6)$$

similarly $b_2 = d_1(W-3) + d_2(W^2-9) + d_3(W^3-27)$

Now substituting equations (6) for b_1 and b_2, respectively, in equation (3) the final equation is obtained:

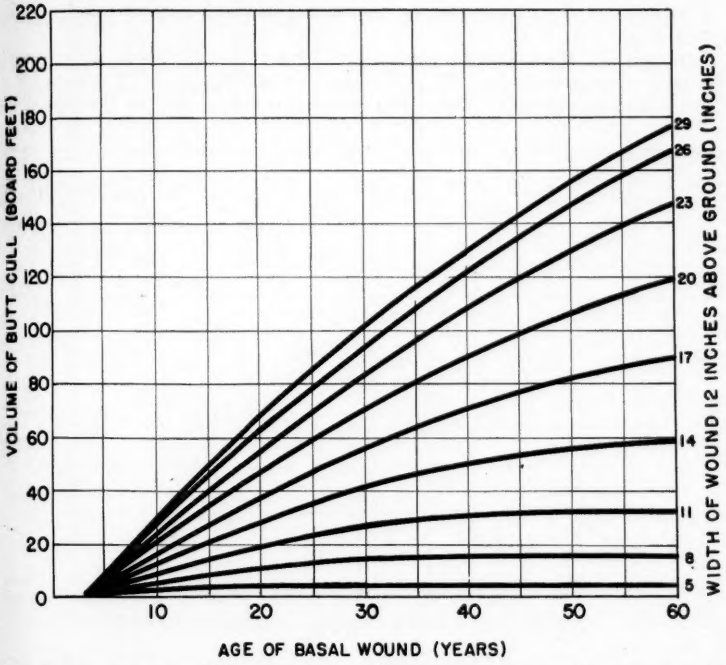

FIGURE 3.—Relation of butt-cull volume in board feet to age of basal wound for different wound widths. These curves were derived by multiple-regression analysis.

$$Y = c_1(W-3)(A-3) + c_2(W^2-9)(A-3) + c_3(W^3-27)(A-3) + d_1(W-3)(A^2-9) + d_2(W^2-9)(A^2-9) + d_3(W^3-27)(A^2-9).$$

The solution of this equation by the method of least squares gave the following for wound ages up to 60 years and widths up to 31 inches:

$$\begin{aligned} Y = {} & 0.1467 \pm 0.02876\ (W-3)\ (A-3) \\ & + .0040456 \pm .0020171\ (W^2-9)\ (A-3) \\ & - .000115276 \pm .000052085\ (W^3-27)(A-3) \\ & - .0043127 \pm .0005517\ (W-3)(A^2-9) \\ & + .000223 \pm .0000415\ (W^2-9)\ (A^2-9) \\ & + .0000038365 \pm .0000010952\ (W^3-27)\ (A^2-9) \end{aligned}$$

The equation is graphically illustrated in figure 3. In the development of the equation, instead of computing squares and cross prod-

ucts for each tree, the data were grouped into nine classes of wound width and seven classes of age. The equation was derived from the class means, together with their appropriate frequencies. The standard error, computed directly from the residuals for each tree, is 48, which is 112 percent of mean Y. The equation accounts for 61 percent of the sum of squares of cull around zero. The magnitude of the standard error varies with estimated Y, as shown in figure 4. This regression (fig. 4) was determined by grouping the trees into classes of estimated Y and computing the standard deviation of residuals for each class.

In figure 5 the residuals are plotted about the net regression curves of cull over wound width and cull over wound age. These curves show that the equation type adopted is suitable and that the equation

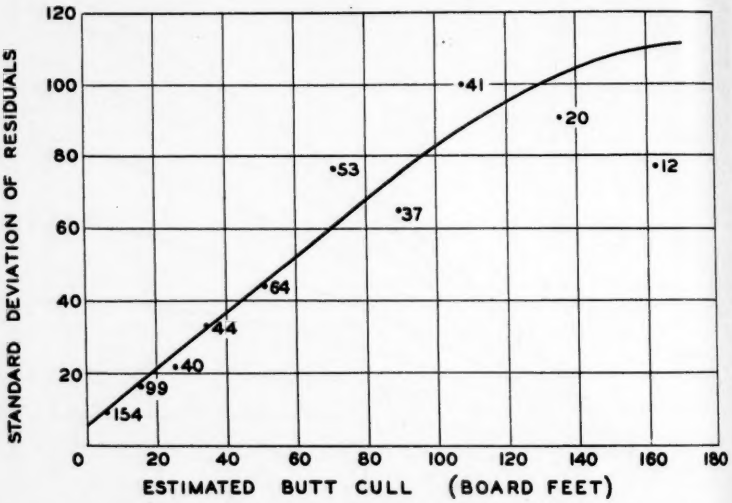

FIGURE 4.—Variation of the standard deviation of residuals with volume of butt cull as estimated from the regression equation. The numbers beside the points indicate the number of trees on which each point is based.

satisfies the data reasonably well. The unaccountably large amount of cull in the trees with wounds in the 40-year age class raised the general level of the curve in figure 5, *B*, above that defined by the other age classes.

The estimates of cull from figure 3 are not in close agreement with those of Hepting and Hedgcock (*3*, *fig. 6*). While in the earlier work wound age as a variable was not included and the graphs are thus not readily comparable with figure 3 of the present work, nevertheless estimates of cull from the earlier curves would run consistently higher than those from figure 3. The present curves, while yet perhaps not the final answer, should be regarded as superseding those given by Hepting and Hedgcock. Since the earlier work was not designed primarily to study decay behind basal wounds, it is possible that the smaller closed wounds on many of the sound trees were not recorded.

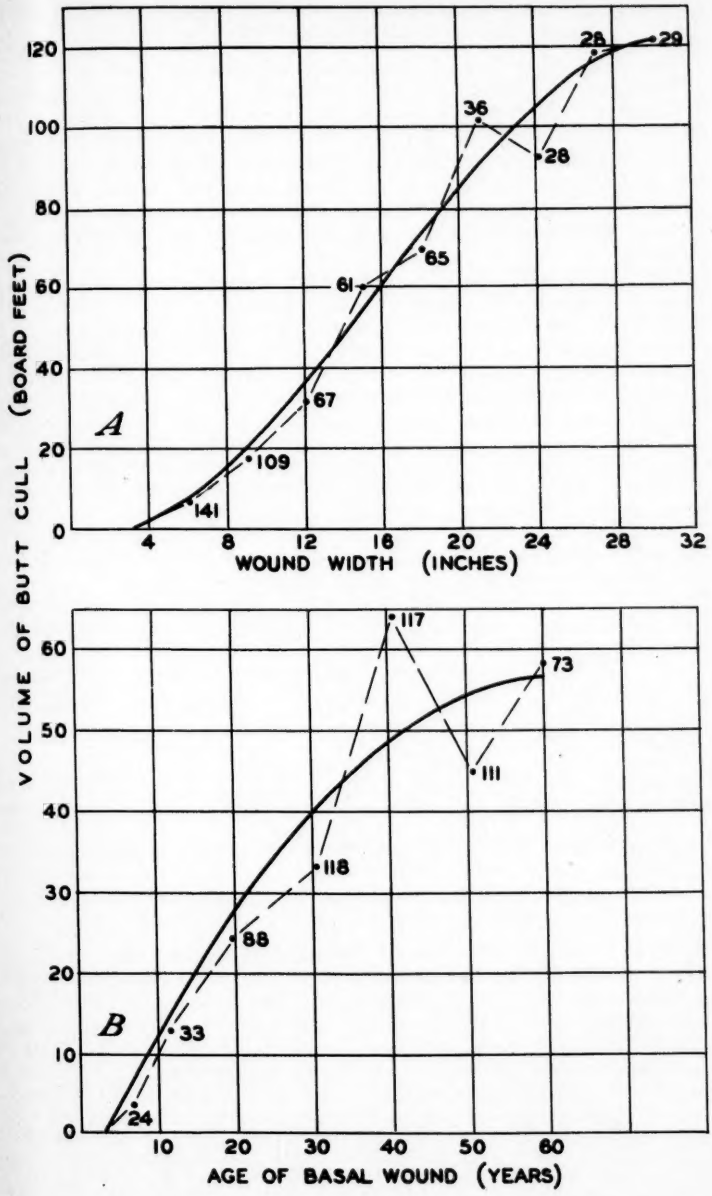

FIGURE 5.—Residuals plotted about the net regression of butt-cull volume over (*A*) wound width, for mean wound age, and (*B*) wound age, for mean wound width. The numbers beside the points indicate the number of trees on which each point is based.

SIGNIFICANCE OF VARIABILITY IN CULL AMONG SPECIES, SITES, AND LOCALITIES

The possibility that mathematically significant differences in rate of decay existed among the five species of oak studied was tested by the analysis of variance of residuals after reducing them to standard units. The residual for each tree was divided by the standard deviation of residuals appropriate to its estimated cull, from figure 4, thereby putting the residuals in terms of standard units. Thus, the variances of residuals at different points along the regression lines covered essentially the same range and could be pooled into a general analysis.

Differences among species were tested separately for wounds up to 12 inches wide and for those 13 inches wide and over. In table 1 the residuals, by species and by two classes of wound width, are given. In neither case was the mean square among species significant at the 5-percent level by Snedecor's F test (*6*). Table 2 presents the analysis of variance for all wound widths combined. The failure of the mean square among species to appear significant does not necessarily mean that these five species decay at the same rate, but it means that from these data it cannot be demonstrated that differences exist within a reasonable degree of expectancy, because the variation in rate of decay within a species was as great as among species.

TABLE 1.—*Mean residuals for the 5 species of oak, by wound-width classes, in terms of standard units* [1]

Species	Wounds 4 to 12 inches wide		Wounds 13 to 31 inches wide		Total wounds	
	Number	Mean residual (standard units)	Number	Mean residual (standard units)	Number	Mean residual (standard units)
White oak	106	−0.15	134	+0.18	240	+0.03
Chestnut oak	84	−.11	68	−.08	152	−.10
Red oak	52	+.02	25	−.14	77	−.04
Black oak	40	−.13	32	−.30	72	−.20
Scarlet oak	20	+.01	3	+.03	23	+.01

[1] A plus sign indicates a mean cull volume greater than, and a minus sign, less than, estimated cull based on all species.

TABLE 2.—*Analysis of variance in cull among 5 species of oak*

Variance	Degrees of freedom	Sum of squares	Mean square	Odds that the factor is significant
Among species	4	3.7495	0.9374	<20 : 1
Within species	559	561.3608	1.0042	
Total	563	565.1103		

The method used to test differences in decay rate among the species of oak was also used to test the effect of site. The following mean residuals, expressed in standard units, indicated that site might have a significant effect: Stream bottom [4] −0.17, lower slope −0.11, middle

[4] Stream bottom includes land within 10 feet elevation of running water; ridge includes land within 10 feet elevation of a ridge top. The slope between stream bottom and ridge is equally divided into lower, middle, and upper slope. This method of expressing site was suggested by L. I. Barrett, of the Appalachian Forest Experiment Station, Forest Service, U. S. Department of Agriculture.

slope −0.06, upper slope −0.03, and ridge +0.11. The analysis of variance presented in table 3, however, fails to show the differences in decay rates among sites as significant. There is ample evidence that susceptibility to wounding by fire varies considerably among the species of oak (*4*) and among sites, but it is not possible to demonstrate through this investigation that wounds of the same size on the different oaks studied or on different sites will result in different amounts of cull.

TABLE 3.—*Analysis of variance in cull among 5 site qualities*

Variance	Degrees of freedom	Sum of squares	Mean square	Odds that the factor is significant
Among sites	4	2.4556	0.6139	<20 : 1
Within sites	559	562.6023	1.0064	
Total	563	565.0579		

The significance of differences in cull among the 11 study areas was tested in a manner similar to the tests for species and site. The mean residuals for the different areas, expressed in standard units, ranged from −0.18 to +0.43. The analysis of variance (table 4) fails to show the differences in cull among localities to be significant. Certain individual differences in cull between areas with the most and the least cull were significant, but in general the differences were so small that the mean square among localities failed to show significance.

TABLE 4.—*Analysis of variance in cull among 11 localities*

Variance	Degrees of freedom	Sum of squares	Mean square	Odds that the factor is significant
Among localities	10	17.0051	1.7005	<20 : 1
Within localities	553	548.1317	0.9912	
Total	563	565.1368		

FUNGI ISOLATED FROM BUTT DECAY

Perhaps the most important single factor contributing to the great variation in butt-cull volume among trees of the same species and with wounds of approximately the same size and age is the fungus succession in the wood behind the wounds. During the first few years the fruiting bodies of numerous fungi that rot dead sapwood commonly appear on the dead bark over basal wounds. During the first summer after a fire *Schizophyllum commune* Fr., *Panus stipticus* Bull. ex Fr., *Daldinia concentrica* (Bolt ex Fr.) Ces. and De Not., *Nummularia* sp., and an occasional *Stereum* or *Polyporus* commonly appear; the second year *Polyporus versicolor* L. ex Fr., *P. pargamenus* Fr., *Stereum rameale* Schw. ex Burt, *S. lobatum* Fr., and *Lenzites betulina* L. ex Fr. are the most common that occur; and by the third year *P. gilvus* Schw. ex Fr.

usually makes its appearance. These fungi are generally followed by the heart rotters, which rarely signify their presence by producing sporophores. In some cases, especially behind small wounds, the sap rot fungi will progress a short distance into the heartwood and no further decay will follow.

Apparently chance has much to do with which heart rot fungi become established behind any given wound in oaks. During this study cultures were prepared from the decaying wood behind a number of wounds where specimens could be obtained that gave promise of yielding pure cultures.[5] Table 5 includes a list of the fungi isolated and the maximum and the average height above ground that the decays extended. Although table 5 is based upon isolations from wounds of different widths and ages, it strongly suggests that the extent of decay is partly dependent upon the fungus involved. This had previously been demonstrated for a somewhat different group of fungi in the case of southern bottom-land hardwoods (*2*).

TABLE 5.—*Fungi isolated from butt decay*

Group [1]	Fungus	Isolations	Height of evident decay	
			Maximum	Average
		Number	*Feet*	*Feet*
1	*Hydnum erinaceus* Bull. ex Fr.	14	21.0	8.8±1.36
	Stereum frustulosum Pers. ex Fr.	22	18.6	8.7± .83
	Poria cocos (Schw.) Wolf	4	8.3	7.4± .40
	Polyporus berkeleyi Fr.	8	8.8	6.7± .63
	Polyporus frondosus Dicks. ex Fr.	4	7.7	6.3± .69
	Armillaria mellea Vahl ex Fr.	4	5.3	4.2± .48
2	*Poria andersonii* (Ell. and Ev.) Neuman	2	25.2	16.2
	Polyporus spraguei Burk. and Curt.	1	16.0	16.0
	Polyporus dryophilus Berk.	2	28.7	14.6
	Polyporus dryadeus Pers. ex Fr.	2	15.9	10.5
	Corticium lividum Pers. ex Fr.	1	10.5	10.5
	Polyporus zonalis Berk.	2	13.2	10.3
	Poria nigra Berk.	3	10.0	8.7
	Polyporus sulphureus Bull. ex Fr.	2	7.5	6.0
	Polyporus pseudo-sulphureus Long	1	5.3	5.3
	Fistulina hepatica Huds. ex Fr.	1	3.7	3.7

[1] Group 1 includes only those fungi that are represented by 4 or more isolates, and for them the standard error of the average decay height is given; group 2 includes those fungi represented by less than 4 isolates, and for them no standard errors were computed.

PREDICTION OF CULL

By the end of one growing season after a fire the limits of the basal wounds formed can be fairly accurately determined because of the raised callus ridges at the edges of the wounds and the dead, loosened bark. A tally of the new basal wounds according to their widths 12 inches above ground and the application of the equation illustrated in figure 3 afford a means of predicting the volume of cull that will follow. If the number of trees wounded and the wound widths are known, together with the approximate date of harvest, the expected board-foot loss at the time of harvest can be computed. If this volume is converted to dollars per acre, and the latter value discounted to the time of the fire at an appropriate interest rate, an estimate of the financial loss due to cull can be obtained. The greater the number of trees wounded, the greater the accuracy of prediction.

[5] Cultures were prepared from the specimens and determinations were made by R. W. Davidson, of the Divison of Forest Pathology.

For example, cull predictions for a group of 500 trees would have, under average conditions, a standard error of about 5 percent, and for 1,000 trees, 3 percent.

Basal wounds should be tallied only on those trees that are already crop trees or that show fair prospect of becoming such. Hence no cull would be computed for the overtopped and part of the intermediate trees or trees already culls when wounded. Neither should wounds be tallied on trees already known to be butt-rotted. The examination of all crop trees on a burned area would ordinarily be unnecessary. Except in the case of very small fires, a 5- or 10-percent tally, by strips or plots, would probably suffice to sample the extent of wounding.

The integration of the fire losses due to cull, mortality, and other factors, leading to a complete analysis of damage from individual fires expressed in dollars per acre, is the goal of the series of investigations of which this is a part. A recent article by Bentley (*1*) illustrates the need for sound standardized methods of predicting fire damage in hardwoods, so that personal judgment will be reduced to a minimum. He cites a specific case in a cut-over hardwood stand in Tennessee, where the damage resulting from certain fires was greatly underestimated. His own method of damage appraisal, however, appears to lack factual basis and to provide estimates much too high.

SUMMARY

Investigation of decay that developed from basal fire wounds on oaks led to the development of a statistical mechanism by which the cull that follows a particular fire can be predicted. The predicting equation is for single trees and is based upon the width of basal wounds 12 inches above ground and the number of years since the fire. The data were collected on trees cut for lumber, cross ties, and staves at 11 localities in the Appalachian Mountains.

It could not be demonstrated that basal wounds of equal width produced significantly different amounts of cull among the species of oak, among the different sites, or among the study areas.

Much of the variation in amount of cull among trees with wounds of similar sizes and ages was due to the different fungi that became established. Of the 16 species of decay fungi isolated from decay following wounding, the most common were *Stereum frustulosum* and *Hydnum erinaceus*.

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HYBRIDIZATION BETWEEN USTILAGO AVENAE AND U. PERENNANS[1]

By C. S. HOLTON, *associate pathologist, Division of Cereal Crops and Diseases,* and G. W. FISCHER, *associate pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Numerous cases of hybridization between species (*1, 4, 5, 6, 7*)[2] and between genera (*8*) of the smut fungi have been reported in recent years. It is notable, however, that in all such instances the hybrids were produced by inoculating a host known to be common to the organisms involved in the cross. Thus far there has been no report of the production of hybrid chlamydospores in the smut fungi in which the species or genera involved in the cross did not have a common host. Recently, the writers have obtained results that seem to indicate definitely that hybrid chlamydospores were produced by crossing *Ustilago avenae* (Pers.) Jens. (loose smut of oats) and *U. perennans* Rostr. (loose smut of tall oatgrass (*Arrhenatherum elatius* (L.) Mert. and Koch)), two species for which no common host is known. These results are presented herein.

MATERIAL AND METHODS

All cultures used in these investigations were of monosporidial origin and were obtained by isolating single sporidia from individual, germinating chlamydospores. Each chlamydospore from which sporidia were isolated was given a number and the four sporidia were numbered according to their respective positions on the promycelium, beginning at the distal end from the spore. For example, *Ustilago avenae* 52–1, 52–2, 52–3, 52–4 are the designations for monosporidial lines obtained from chlamydospore 52 of that species. The sex reaction of the various groups of monosporidial lines was determined by mating them in all possible combinations of two on plain agar and examining for sporidial fusions after 24 hours or longer. Only those combinations showing sporidial fusions in culture were used for inoculation purposes. In some of the tests the inoculum was composed of only two monosporidial lines, in which case the combination is indicated as 1×2 or 3×4, as the case may be, while in other instances the inoculum was composed of four lines, two of one sex and two of the opposite sex, in which case the combination is indicated as $1+2\times3+4$. In the case of an interspecies combination the culture number or numbers shown first represent the species listed first. For example, *U. avenae* $54\times$*U. perennans* 8 sporidial combination 1×3 denotes that culture No. 1 of *U. avenae* 54 was combined with culture No. 3 of *U. perennans* 8.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 128.

The culture inoculum was prepared by mixing sporidia of opposite sex on 2-percent potato-dextrose agar and incubating at room temperature for 12 to 18 hours, at which time a sporidial suspension of the combined cultures was made in sterile tap water. Chlamydospore inoculum consisted of a suspension of spores in tap water made immediately before inoculation. Inoculations were made by the partial-vacuum method described by Allison (*1*). The oat seed was soaked in formaldehyde solution (1:320) for 10 minutes to kill any smut that might be present as a result of field inoculation and it was then washed with running water to remove the formaldyhyde. The tall oatgrass seed was given the same formaldehyde treatment for 1 hour.

RESULTS

PATHOGENICITY OF INTRASPECIES AND INTERSPECIES HYBRIDS

Seed of Victory oat (C. I.[3] 560) (*Avena sativa* L.) and wild oats (*A. fatua* L.) was inoculated with intraspecies and interspecies sporidial combinations of *Ustilago avenae* and *U. perennans* and planted in the greenhouse in the winter of 1937–38. Both hosts were infected by *U. avenae*, neither host was infected by *U. perennans*, and slight infection was obtained on wild oats only with the interspecies combinations. The interspecies F_1 chlamydospores were echinulate, resembling both parents in this respect. Furthermore, they were capable of normal germination, but the sporidia, with few exceptions, were incapable of growth on culture media.

In view of these preliminary results, the inoculations were repeated and the seed was planted in the field in the spring of 1938. The results, presented in table 1, substantiate those obtained from the preliminary inoculations. *Ustilago avenae* produced 60 and 90 percent smut on Victory and wild oats, respectively, while *U. perennans* produced no smut on either of these hosts. The two interspecies crosses failed to infect Victory oats but produced slight infection (0.8 and 3.2 percent) on wild oats. As shown in table 1, no results were obtained from the inoculations of tall oatgrass because the plants did not head. Again it was found that the hybrid chlamydospores produced on wild oats germinated normally, but the sporidia would not grow in culture.

The F_1 chlamydospores of the interspecies crosses were used to inoculate seed of the three hosts which was planted in the greenhouse in the fall of 1938. The results of these inoculations are also presented in table 1. Both Victory oats and wild oats were infected by the two hybrids, one hybrid producing 96 and 92 percent smut and the other 38.5 and 16.5 percent smut on these two hosts, respectively. In contrast, tall oatgrass was not infected by either hybrid. The failure to recover a *Ustilago perennans* segregate on tall oatgrass is difficult to explain, especially in view of the fact that a *U. avenae* segregate apparently was recovered on Victory oats. Furthermore, the evidence that *U. perennans* actually was one parent of the two hybrids is entirely of the negative type, being based on the fact that Victory oats and wild oats, both of which are highly susceptible to *U. avenae*, were not infected by the *U. perennans* inoculum. However, a new series of inoculations on seed planted in the greenhouse in the fall of 1938 gave results that substantiate those described above.

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

TABLE 1.—*Smut resulting from inoculations with intraspecies and interspecies sporidial combinations and F_1 hybrid chlamydospores of Ustilago avenae × U. perennans*

Species and cross	Sporidial combination	Inoculum	Smutted panicles in—		
			Victory oats	Wild oats	Tall oatgrass
			Percent	*Percent*	*Percent*
U. avenae 52	1+4 × 2+3	Sporidia	60.0	90.0	(1)
U. perennans 5	1+3 × 2+4	do	0.0	0.0	(1)
U. avenae 52 × *U. perennans* 5	1+4 × 2+4	do	0.0	0.8	(1)
	2+3 × 1+3	do	0.0	3.2	(1)
	1+4 × 2+4	F_1 chlamydospores	96.0	92.0	0.0
	2+3 × 1+3	do	38.5	16.5	0.0

[1] Inoculations were made, but the plants failed to head.

In the second series of inoculations, cultures of two races of *Ustilago avenae* and of one race of *U. perennans* were used and the inoculum in each case was composed of compatible combinations of two monosporidial lines, both within a species and between species. Inoculations were made on Anthony oats, wild oats, and tall oatgrass, except that tall oatgrass was not inoculated with *U. avenae*, and Anthony oats were not inoculated with *U. perennans* and the interspecies combinations. The results of these inoculations are presented in table 2. The efficiency of the inoculation process and the fact that optimum conditions for infection prevailed are well established by the results obtained with Anthony oats. Six of the eight sporidial combinations of *U. avenae* used to inoculate this variety produced 100 percent infection and the other two produced more than 90 percent infection. The susceptibility of wild oats to the *U. avenae* inoculum was shown by the fact that one of the races produced 83 percent and the other race 82 percent infection on this host. The inoculation of tall oatgrass and wild oats with *U. perennans* resulted in infection of the former by all four sporidial combinations used, the estimated percentages ranging from 15 to 35, while no smut was produced on wild oats by any of the inoculations with this species.

In table 2 it can be seen that of the 16 interspecies combinations used to inoculate wild oats and tall oatgrass, 4 produced smut on the former while none produced smut on the latter. Sporidium No. 1 of *Ustilago avenae* 54 crossed with sporidium No. 3 of *U. perennans* 8 produced 83.3 percent smut on wild oats and sporidium No. 4 crossed with sporidium No. 3, of the same two species, respectively, produced 53.8 percent smut on wild oats. Likewise the same sporidial combinations, i. e., 1×3 and 4×3, of *U. avenae* 56 and *U. perennans* 8, produced 10 percent and 18.1 percent smut, respectively, on wild oats. In this connection it should be noted, as shown in table 2, that the 4 interspecies combinations in which the sporidia fused and subsequently produced infection hyphae in culture produced smut on wild oats, while no infection resulted from the inoculations with those sporidial combinations in which the sporidia fused without the subsequent production of infection hyphae. Thus, apparently there were two degrees of compatibility in the interspecies combinations of opposite sex, represented on the one hand by sporidial fusions

without further development in culture, and on the other hand by sporidial fusions followed by the production of infection hyphae and ultimately the development of chlamydospores on the host.

TABLE 2.—*Smut resulting from inoculations with intraspecies and interspecies crosses of Ustilago avenae and U. perennans*

Species and cross	Sporidial combination	Sex reaction in culture [1]	Smutted plants [2] in—		
			Anthony oats	Wild oats	Tall oatgrass
			Percent	*Percent*	*Percent*
U. avenae 54	1 × 2	++	100.0	83.0	.....
	1 × 3	++	100.0	.....	.....
	2 × 4	++	100.0	.....	.....
	3 × 4	++	91.0	.....	.....
U. avenae 56	1 × 2	++	95.0	82.0	.....
	1 × 3	++	100.0	.....	.....
	2 × 4	++	100.0	.....	.....
	3 × 4	++	100.0	.....	.....
U. perennans 8	1 × 3	++	.....	0.0	30.0
	1 × 4	++	.....	0.0	15.0
	2 × 3	++	.....	0.0	35.0
	2 × 4	++	.....	0.0	30.0
U. avenae 54 × *U. perennans* 8	1 × 3	++	.....	83.3	0.0
	1 × 4	+	.....	0.0	0.0
	2 × 1	+	.....	0.0	0.0
	2 × 2	+	.....	0.0	0.0
	3 × 1	+	.....	0.0	0.0
	3 × 2	+	.....	0.0	0.0
	4 × 3	++	.....	53.8	0.0
	4 × 4	+	.....	0.0	0.0
U. avenae 56 × *U. perennans* 8	1 × 3	++	.....	10.0	0.0
	1 × 4	+	.....	0.0	0.0
	2 × 1	+	.....	0.0	0.0
	2 × 2	+	.....	0.0	0.0
	3 × 1	+	.....	0.0	0.0
	3 × 2	+	.....	0.0	0.0
	4 × 3	++	.....	18.1	0.0
	4 × 4	+	.....	0.0	0.0

[1] The double plus sign (++) indicates sporidial fusions with the subsequent production of infection hyphae, while the single plus sign (+) indicates sporidial fusions without the subsequent production of infection hyphae.

[2] Based on plant counts, the total number ranging from 15 to 24 in Anthony oats and 9 to 15 in wild oats, except in tall oatgrass, where the percentages were estimated.

In view of the apparent difference in degree of compatibility of the interspecies sporidial combinations, another inoculation test was made in which only those combinations were used for inoculum in which fused sporidia produced infection hyphae, and only one host, wild oats, was inoculated. In addition, the F_1 chlamydospores of the interspecies hybrids shown in table 2 were used to inoculate Anthony oats and wild oats, which were seeded in the field in the spring of 1939. The results are presented in table 3. The susceptibility of the wild oats to the *Ustilago avenae* inoculum is shown by the production of 85, 80, 50, and 77.6 percent smut by the four combinations of monosporidial lines used. On the other hand, *U. perennans* produced no smut on this host. All of the sporidial combinations involving *U. avenae* 54, 56, and 57 with *U. perennans* 8 produced smut, the percentages ranging from 2.5 to 90. Of the four sporidial combinations between *U. avenae* 55 and *U. perennans* 8, only one, 4×3, produced smut (47.2 percent). There is no apparent explanation for the failure of the other three combinations to produce smut, as infection hyphae were produced by the fused sporidia on plain agar. Likewise it is difficult to explain the wide differences in the amounts of smut produced by other interspecies combinations shown in table 3. It is hoped that further studies will make possible an explanation of these results.

The results of the inoculations with F_1 chlamydospores of the four interspecies hybrids show a high degree of infection (50 to 95.2 percent) with three of the hybrids, and low percentages of infection (7.5 to 7.9 percent) with the other hybrid (table 3). Thus, segregates having a pathogenicity similar to that of the *Ustilago avenae* parent were recovered on Anthony oats in the F_2 generation, although no smut was produced on this variety in the F_1 generation (table 2). The results of inoculations on tall oatgrass with these four hybrids are under investigation.

TABLE 3.—*Smut resulting from inoculations with intraspecies and interspecies sporidial combinations and F_1 hybrid chlamydospores of Ustilago avenae and U. perennans*

Species and cross	Sporidial combination	Sex reaction in culture	Inoculum	Smutted panicles[1] in—	
				Anthony oats	Wild oats
				Percent	*Percent*
U. avenae 54	1+4×2+3	++	Sporidia		85.0
U. avenae 56	1+4×2+3	++	do		80.0
U. avenae 55	1+2×3+4	++	do		50.0
U. avenae 57	1+3×2+4	++	do		77.6
U. perennans 8	1+2×3+4	++	do		0.0
U. avenae 54×*U. perennans* 8	1×3	++	do		46.7
	4×3	++	do		86.0
U. avenae 56×*U. perennans* 8	1×3	++	do		83.3
	4×3	++	do		16.4
U. avenae 57×*U. perennans* 8	3×1	++	do		4.2
	3×2	++	do		2.5
	4×3	++	do		90.0
	4×4	++	do		3.3
U. avenae 55×*U. perennans* 8	1×1	++	do		0.0
	1×2	++	do		0.0
	4×3	++	do		47.2
	4×4	++	do		0.0
U. avenae 54×*U. perennans* 8	1×3		F_1 chlamydospores	62.1	92.3
	4×3		do	50.0	95.2
U. avenae 56×*U. perennans* 8	1×3		do	7.9	7.5
	4×3		do	61.1	78.1

[1] Based on total panicles in each 5-foot row in the field.

COMPATIBILITY OF MONOSPORIDIAL COMBINATIONS

The difference in degree of compatibility between the combinations of monosporidial cultures previously described is substantiated by results obtained with combinations of two other monosporidial complements of *Ustilago perennans* with *U. avenae*, *U. levis* (Kell. and Sw.) Magn., a buff strain of *U. levis*, and *U. hordei* (Pers.) Kell. and Sw.

Ustilago perennans 5 and 6, each represented by full complements of four pedigreed monosporidial cultures, exhibited nothing unusual when mated with each other in all possible combinations, as shown in table 4. The eight monosporidial lines all seemed equally able to fuse with others of opposite sex, and fusion was followed by the normal development of infection hyphae. However, when these same eight cultures were paired with cultures of the other species, a compatibility difference, which is linked with the sex involved, became evident. The results of these matings are recorded in table 5.

In each of the two complements of pedigreed monosporidial cultures of *Ustilago perennans* there are two cultures that are more active in interspecies matings than the other two, and, also, in each case the two stronger cultures are of the same sex. In *U. perennans* 5, it is

cultures 1 and 2 in each instance that participate in the development of infection hyphae with whatever sporidia of opposite sex they are paired. Sporidia of the other two cultures, 3 and 4, fused with sporidia of opposite sex of the other species, but the union was seldom followed by the production of infection hyphae. In *U. perennans* 6, cultures 3 and 4 were the more active, and here again it is notable that these two are of the same sex. Furthermore, it is seen that in *U. perennans* 6, cultures 3 and 4 are of the same sex as *U. perennans* 5, cultures 1 and 2, as indicated by the results of cross-pairing these cultures.

TABLE 4.—*Normal reaction obtained when two complements of pedigreed monosporidial cultures of Ustilago perennans are paired with each other on plain water agar*

[++ = infection hyphae produced by fused sporidia; − = no reaction]

Chlamydospore No.	Sporidial No.	Sporidial No.							
		U. perennans 5				*U. perennans* 6			
		1	2	3	4	1	2	3	4
U. perennans 5	1	−	−	++	++	++	++	−	−
	2	−	−	++	++	++	++	−	−
	3	++	++	−	−	−	−	++	++
	4	++	++	−	−	−	−	++	++
U. perennans 6	1	++	++	−	−	−	−	++	++
	2	++	++	−	−	−	−	++	++
	3	−	−	++	++	++	++	−	−
	4	−	−	++	++	++	++	−	−

TABLE 5.—*Anomalous sex reaction obtained when two complements of pedigreed monosporidial cultures of Ustilago perennans were paired with U. avenae, U. levis, and U. hordei on plain water agar*

[++=infection hyphae produced by fused sporidia; +=fusions, but no infection hyphae produced; −=no fusions]

Chlamydospore No.	Sporidial No.	Sporidial No.							
		U. perennans 5				*U. perennans* 6			
		1	2	3	4	1	2	3	4
U. avenae 33	1	++	++	−	−	−	−	++	++
	2	−	−	+	+	+	+	−	−
	3	−	−	+	+	+	+	−	−
	4	++	++	−	−	−	−	++	++
U. levis 77	2	++	++	−	−	−	−	++	++
	3	−	−	+	+	+	+	−	−
	4	−	−	+	+	+	+	−	−
U. levis buff 47	1	++	++	−	−	−	−	++	++
	2	++	++	−	−	−	−	++	++
	3	−	−	+	+	+	+	−	−
	4	−	−	+	+	+	+	−	−
U. levis buff 50	1	−	−	+	+	+	+	−	−
	2	++	++	−	−	−	−	++	++
	3	++	++	−	−	−	−	++	++
	4	−	−	+	+	+	+	−	−
U. hordei 5	1	++	++	−	−	−	−	++	++
	2	++	++	−	−	−	−	++	++
	3	−	−	+	+	+	+	−	−
	4	−	−	+	+	+	+	−	−

It seems apparent from the results given above that three types of reaction are manifest when monosporidial cultures of *Ustilago perennans* are mated with monosporidial lines of certain other *Ustilago* spp.: (1) Negative reaction, resulting from pairing two cultures of the same sex; (2) fusion between pairs of sporidia of opposite sex, and the subsequent development of infection hyphae; and (3) fusion of sporidia of opposite sex, but not followed by the production of infection hyphae. Although the last two types of reaction result from pairing cultures of opposite sex, apparently there also is involved some difference in the degree of compatibility between the two sexes. Similar differences were noted by Fischer (*3*) when monosporidial cultures of *U. striaeformis* (Westd.) Niessl. were paired with monosporidial cultures of *U. bullata* Berk. Bauch (*2*) reported much the same phenomenon in *Sphacelotheca schweinfurthiana* (v. Thüm.) Sacc., in which case intraspecies matings exhibited different types of reaction: in one type ("W-Reaktion") no further development of the fused sporidia took place, whereas in the other ("S-Reaktion") binucleate infection hyphae developed from the fused sporidia in the normal manner.

The basis for this anomalous sexual behavior is very difficult to determine. Some of the interspecies sporidial combinations were more compatible than others, and furthermore it was always the same two cultures in each set of four that were active enough to participate in the formation of infection hyphae. Perhaps it is significant that in each case the sporidia of the two more active cultures of a complement of four were of the same sex, not only with reference to their sister cultures but also with reference to the two more active cultures of another complement of four. The phenomenon is made still more complicated by the fact that in intraspecies matings between cultures of *Ustilago perennans* these compatibility differences have not been observed.

SUMMARY

Ustilago avenae and *U. perennans*, which do not have a known common host, were hybridized by inoculating wild oats with compatible monosporidial combinations of the two species. The wild oats were infected, but no infection was obtained from similar inoculations on Victory oats and tall oatgrass. Inoculations with *U. avenae* produced infection on Victory oats and wild oats but not on tall oatgrass, while *U. perennans* infected tall oatgrass but not Victory oats or wild oats.

Segregates having a pathogenicity similar to that of the *Ustilago avenae* parent were recovered from the hybrid chlamydospores by inoculating Anthony oats, but segregates behaving like the *U. perennans* parent were not recovered from similar inoculations on tall oatgrass.

When the sexually opposite monosporidial lines of *Ustilago perennans* were paired with *U. avenae*, *U. levis*, and *U. hordei*, two different degrees of compatibility were exhibited, viz, fusion of sporidia followed by the production of infection hyphae and fusion of sporidia without the subsequent production of infection hyphae. Infection of wild oats was produced by the former type, but not by the latter.

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